

## REVIEW

# Pathogenetics. An introductory review



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### KEYWORDS

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**Abstract** Pathogenetics refers to studying the different aspects of initiation/development/progression and pathogenesis of genetic defects. It comprises the study of mutagens or factors capable of affecting the structural integrity of the genetic material leading to mutational changes that, in the majority of cases, result in harmful effects due to the resulting disturbances of functions of mutated components of the genome. The study of mutagens depicts different types of mutagenic factors, their nature, their classification according to their effects on the genetic material and their different modes of action. The study of mutation involves different types of mutations classified according to various parameters, e.g. magnitude, severity, target of mutational event as well as its nature, which can be classified, in turn, according to whether it is spontaneous or induced, static or dynamic, somatic or germinal mutation etc. Finally, pathogenetics comprises studying and delineating the different and innumerable pathophysiological alterations and pathogenetic mechanisms that are directly and indirectly involved in, and leading to, the development of genetic disorders, coupled with a parallel study of various anti-mutation mechanisms that play critical roles in minimizing the drastic effects of mutational events on the genetic material and in effective protection against the development of these diseases.

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## 1. Definition

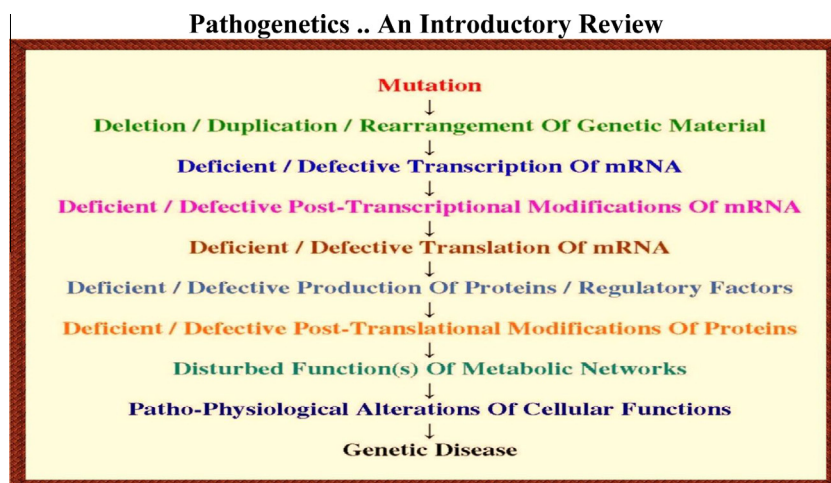
Pathogenetics encompasses studying the various factors and different mechanisms involved in the pathogenesis of genetic diseases. It comprises the study of mutagens or factors that can cause change and/or damage of the genetic material, the study of mutations or acquired structural/functional changes of the genetic components at any of its organizational levels that result secondary to the effects of mutagens, the study of the various anti-mutation mechanisms of the human genome/transcriptome/proteome that exert an essential and pivotal role in maintaining the integrity/stability/identity of the genome, the study of the different and varying pathophysiological alterations in cellular functions secondary to the ensuing disturbances of the metabolic-regulatory networks that mediate and control these functions, and the study of the different pathogenetic mechanisms that mediate the development and pathogenesis of genetic defects due to the ensuing changes of the normal structure/function framework of the genetic material.

## 2. Pathogenesis of genetic diseases

Genetic diseases are caused by harmful mutations. Mutations cause structural changes and consequent functional alterations of the affected components of the genetic material, leading

ultimately to a deficient synthesis of proteins or synthesis of defective proteins necessary for mediation of cellular activities. These functional consequences are reflected in disturbed/defective construction of one or more of the metabolic networks that regulate every aspect of cellular functions. As depicted from the cardinal dogma of molecular biology, mutated genome results in the transcription of altered transcriptome which, in turn, causes synthesis of a defective proteome. The defective proteome leads to wide spread pathophysiological alterations that pave the way for the development of various pathogenetic mechanisms responsible for initiation and pathogenesis of genetic disorders. The spectrum of pathophysiological alterations and pathogenetic mechanisms is quite wide and is steadily expanding as more knowledge of the structure/function/behavior/regulation of the genetic material is revealed. However, the basic etiological outline of development of genetic defects, as depicted in [Fig. 1](#), can be summarized as follows ([Fig. 1](#)):

- (1) Deletion or loss of part of a gene, one or many genes, part of a chromosome, one or more chromosomes, one or more of mitochondrial genes, or even a whole genome.
- (2) Duplication/rearrangement of the genetic material.
- (3) Deficient/defective transcription of mRNA.
- (4) Deficient/defective post-transcriptional modifications of mRNA.



**Figure 1** Pathogenesis of genetic diseases.

- (5) Deficient/defective translation of mRNA leading to deficient/defective production of gene products.
- (6) Deficient/defective post-translational modifications/trafficking of synthesized proteins.
- (7) Deficient/defective synthesis of genetic regulatory components that include transcription nucleoproteins, transcription factors, microRNA, etc.

Irrespective of the site, type, nature or magnitude of the mutational event(s) that drastically affect the genetic material, the resultant alterations in gene function(s) trigger many disturbances in one or more of the cellular metabolic regulatory networks mediated by the deficient/defective gene products, thus leading to a wide and varied spectrum of pathophysiological changes in cellular functions leading, ultimately, to the development of genetic diseases. The specific pathognomonic phenotype that characterizes each genetic disease is primarily determined by the spectrum of pathophysiological changes in affected subjects. These, in turn, are determined by the spectrum of the mutation-induced damage to the genetic material in affected patients.

### 3. Mutagens

Mutagens are factors capable of affecting the structural integrity of the genome. The number of harmful mutagens we are continually exposed to, and interacting with, in our life, starting with fertilization and ending by death, is innumerable. However, due to the many lines of defense against the harmful effects of these factors, represented by the many anti-mutation mechanisms of the genome and proteome, only a small percentage of mutational events can reach a threshold level beyond which genetic defects can develop.

### 4. Classification of mutagens

- (A) According to their nature, mutagens are classified into three main categories:
  - (1) Chemical mutagens: these compounds are innumerable in the environment and include, for example, organic compounds, asbestos, insecticides, herbicides, heavy metals, etc.

- (2) Physical mutagens: these include particulate radiations like X ray, alpha particles, UV waves at 2800 Å wavelength, solar radiation, thermal and mechanical agitation of nucleic acids [1].
- (3) Biological mutagens: these include living microorganisms like some viruses: cytomegalovirus, rubella virus and herpes virus.

- (B) According to their pathogenetic effects, mutagens are classified into four main categories:
  - (1) Non-specific Mutagens.
  - (2) Carcinogens are mutagens that induce malignant transformations in affected cells.
  - (3) Clastogens are mutagenic agents that can induce chromosome breaks in affected cells.
  - (4) Teratogens are mutagens that cause congenital malformations in exposed fetuses. (Fig. 2)

The pathogenetic effects of mutagens and the resulting pathological alterations follow disturbed functions of mutated genes. Many types of mutagens have the ability to induce all types of mutagenic damage to the cell. Particulate and non-particulate radiations, for example, are effective carcinogens capable of causing malignant transformation of affected cells. They can also cause chromosomal gaps and breaks. In addition, teratogenic effects in developing fetuses following exposure of pregnant women to radiation are a well-known hazard of overexposure to radiation during pregnancy. This global mutagenic effect is also shared by many other types of mutagens like mutagenic viruses and chemicals that can induce malignant transformation, chromosomal breaks and teratogenic malformations in exposed subjects.

### 5. Factors affecting actions of mutagens

The pathological effects of mutagens are determined by many different factors. Each of these factors has its relative mutagenic potential depending on the circumstances of its action. Congenital malformations caused by teratogens, for instance, depend largely on timing of exposure of the developing

<b>Mutagens : types, effects and examples</b>		
<b>Mutagens</b>	<b>Effects</b>	<b>Examples</b>
<b>Carcinogens</b>	<b>Carcinogenesis and tumor formation.</b>	<b>Chemical : Aflatoxins Biological : Retroviruses Physical : X-ray Irradiation</b>
<b>Clastogens</b>	<b>Chromosome breaks, deletions, rearrangements.</b>	<b>Chemical : Bleomycin Biological : HIV virus Physical : UV waves</b>
<b>Teratogens</b>	<b>Congenital malformations.</b>	<b>Chemical : Valproate Biological : Toxoplasma gondii Physical : X-ray irradiation</b>
<b>Non-specific mutagens</b>	<b>Non-specific damage to the genetic material.</b>	<b>Chemical : Innumerable types Physical : X-ray irradiation Biological : Toxoplasma, Viruses</b>

**Figure 2** Classifications of mutagens.

embryo or growing fetus to their effects. Exposure to teratogens before or after periods of embryonic or fetal growth sensitive to their specific teratogenic effects might result in no or minimal harm. The dose of exposure, whether of a chemical or radiological nature, is also an important factor in determining the resultant mutagenic effects. This dose-threshold effect might act in different synergistic ways. A larger exposure dose will quantitatively cause more damage to wider portions of the genetic material, thus resulting in widespread mutations of the genome. It might also cause damage to genes responsible for repair of mutated DNA, thus preventing repair of mutated genes. In addition, direct non-genetic destructive effects of cellular compartments, of blood supply to cells or of extracellular environment of affected cells will both hasten and increase the resulting damage.

The genetic constitution, or genetic background, of exposed subjects plays a critical role in determining the sensitivity to mutagenic factors as well as the extent of damage following exposure to their effects. The outstanding examples in this respect are the chromosome breakage syndromes, sometimes referred to as DNA or genetic instability syndromes. These diseases include xeroderma pigmentosum, ataxia telangiectasia, Nijmegen syndrome, Bloom syndrome, Fanconi anemia and some other similar diseases. Subjects affected by these genetic disorders have deficient and/or defective DNA repair systems necessary for repairing mutations arising during DNA replication or following exposure to mutagens. Exposure to mutagenic factors in doses that have no effects in normal subjects causes drastic genetic alterations in these patients due to their defective genetic constitution.

## 6. Mutation

Mutation entails any uncoded or unprogramed permanent structural alteration of the genetic material at any of its organizational levels. These levels comprise a spectrum beginning with single nucleotide or a part of the nucleotide (base, sugar, phosphate), DNA, RNA, genes, chromosomes, mitochondrial DNA (mtDNA) up to the whole genome. Mutations may occur without an identifiable cause and are termed spontaneous mutations, or they may occur secondary to exposure to a known cause, and are referred to as induced mutations.

The effects of mutation differ widely according to many factors. These factors include the nature and target of the mutagenic factor causing mutation, the timing and magnitude of the resulting damage, the genotype or the genetic constitution of the affected individual and the balance between synergistic mutagenic effects and anti-mutation mechanisms of the genetic material. The damaging effects of mutation are attributed to the defects they cause in functioning of the mutated genes. Since synthesis of proper gene products, necessary for mediating cellular activities, depends primarily on the integrity of the genetic information embodied within the specific base sequence of the gene, changes or mutation of the exact number or the peculiar arrangement of these bases is expected to result in disturbed gene function. This disturbance might express itself as production of structurally defective gene product, deficient synthesis of enough product or disturbances in the construction of regulatory/metabolic networks responsible for monitoring, harmonizing and controlling cellular functions.

## 7. Classification of mutation

- (1) Induced vs spontaneous mutation
- (2) Nuclear vs mitochondrial mutation
- (3) Somatic vs germinal mutation
- (4) Static vs dynamic mutation
- (5) Pathological vs non-pathological mutation
- (6) Point, small, gross and genomic mutation
- (7) Base, sugar, phosphate group mutation
- (8) Persistent vs reversible mutation [Table 1].

**Table 1** Classification of mutation.

Classification of mutation
1. Induced versus spontaneous mutation
2. Nuclear versus mitochondrial mutation
3. Somatic versus germinal mutation
4. Static versus dynamic mutation
5. Pathological versus non-pathological mutation
6. Point, Small, Gross, Genomic mutation
7. Base, Sugar, Phosphate group mutation
8. Persistent versus reversible mutation



### 7.1. *Induced and spontaneous mutation*

Induced mutations are structural alterations of the genetic material that occur due to exposure to the effects of any of the known mutagens. Spontaneous mutations, on the other hand, are mutations that occur without recognizable causes. Spontaneous mutations happen, probably, on a regular basis because of the intrinsic nature of the genome. Possible potential causes of spontaneous mutation of the genome can be delineated and include: transposon activity, tautomerism and changes of energy states of hydrogen bonds of bases. Transposon activity represents a major potential cause of spontaneous mutations [2]. The movement of transposons results in insertional mutagenesis and disruption of targeted genes or other functional genetic elements. Disturbed gene function with resultant deficiency of gene product, production of defective protein/RNA or loss of genetic regulatory mechanisms exerted by mutated genes follows transposon-induced mutations. Tautomerism of bases of the DNA also accounts for a considerable fraction of spontaneous mutations of the genetic material. Changes of energy states of hydrogen bonds between complementary bases of DNA represent a third potential cause of spontaneous mutation. Alterations resulting from changes in electron resonance and/or proton transfer or relocation between the bonds can lead to tautomeric shift or tautomerization and momentary change of one base to another base, thus inducing point mutation, transition mutation [1]. The actual mechanisms underlying the occurrence of these changes are still largely undefined, precisely. Analysis of DNA base dynamics according to quantum, rather than to Newtonian, principles seems essential for clarifying these mechanisms, and the concept of quantum biology, or studying the structure and function of the genetic material based on principles and rules of quantum mechanics, would certainly prevail due to the progressive increase in our knowledge of the structure and function(s) of our genome and our proteome.

Replicative errors occurring during cell division and replication of the DNA are, arbitrarily, considered as one source of spontaneous mutations. These errors occur, and persist, due to many causes including failure of the proof reading system of DNA replication or failure of the DNA repair system to repair or correct the errors. It is estimated that during every somatic cell division cycle of nearly 20 h duration, about 12,000 point mutations occur along the whole genome. The vast majority of these replicative errors are repaired by the DNA repair system of the cell. If some of these errors escape detection and repair they will persist and be transferred to the genome of daughter cells and become fixed mutations.

### 7.2. *Nuclear and mitochondrial mutation*

Nuclear mutations are mutations that affect the nuclear genome, and mitochondrial mutations are mutations that affect the mitochondrial genome. Mutations of nuclear genes account for the occurrence of most genetic diseases. Due to the marked differences between the specific structural organization of each genome, each type of these mutations has its own characteristic features. Nuclear mutations may affect one or both alleles of a structural gene on an autosome or on a sex chromosome, thus giving rise to any of the traditional genetic disorders: autosomal and sex linked dominant and

recessive diseases. The presence of most nuclear genes as pairs of alleles derived equally from both parents, paternal and maternal alleles, allows for this classification of genetic diseases due to nuclear gene mutations to be formulated clearly. Mitochondrial mutations, on the other hand, have different features due to the peculiar nature of the mtDNA. Each mtDNA molecule consists of 37 genes and exists inside the mitochondrion in large numbers, tens to thousands, depending on the metabolic activities of the cell and its demand for ATP. Thus, the copy number of each mtDNA molecule, and of mitochondrial genes is extremely huge in metabolically active organs, e.g. brain, retina, heart, liver, kidney and skeletal muscles. Mutations of mitochondrial genome can affect a considerable portion of its genes without affecting its efficiency in mediating its functions. It is estimated that mitochondrial dysfunction begins to occur when approximately 80% of its genome gets mutated. This threshold effect does not apply for nuclear mutations where mutation of one single allele can cause a serious disease, as is the case for many autosomal dominant disorders.

Mitochondria in somatic cells are derived from maternal mitochondria in the ovum. The sperm, with rare exceptions, does not offer any mitochondrial genes to the zygote, because sperm mitochondria are present in the neck piece of the sperm, which exists next to its head pro-nucleus and which do not participate in fertilization. Accordingly, mitochondrial disorders result from mutations of maternal mitochondria and are transmitted by carrier or affected mothers to all their offspring. This difference underlies the specific features that characterize the nature and the inheritance pattern of mitochondrial diseases.

### 7.3. *Somatic and germinal mutation*

Somatic mutations refer to mutations of the genome of somatic cells. Since somatic cells do not participate in fertilization or determination of the genetic constitution of the offspring they can't be transmitted to the offspring, i.e. they are not heritable mutations. They can be transmitted only to the daughter cells of mutated cells upon their division. The effects of somatic mutations depend on many factors including the type of cell, the genetic constitution of affected cells, selective targeting of nuclear and/or mitochondrial genome and the mutation burden of the cell. According to the interactive processes involving these factors, somatic mutations might result in cell death if the mutation-induced pathophysiological alterations of the cell exceed its ability to obviate and correct these alterations. Milder alterations can cause deranged cellular function(s) and limited or progressive failure and loss of cellular activities, e.g. progressive organ failure syndrome following overexposure to radiation. Somatic mutations affecting the proto-oncogenes or genes regulating DNA repair systems can result in malignant transformation of the cell and development of cancer.

Somatic cells overburdened with mutation are driven to a special suicidal pathway involving its involuntary death to protect other cells from the hazardous risk of its malignant transformation. It might also be looked at as a cellular economic adaptation behavior by getting rid of mutated diseased, energy consuming and harmful cells. This mechanism of compulsory involuntary or programmed cell death, referred to as apoptosis, represents an important protective anti-mutation

mechanism of the organism to maintain its genomic integrity, and demonstrates one of the basic conservative features of living organisms by executing over mutated cells to prevent spread of their mutations through division to daughter generations.

Germinal mutations are mutations that affect genomes of germ line cells, i.e. ova and sperms, that participate in fertilization and determination of the genetic constitution of the offspring. If the particular ovum or sperm affected by the mutation happens to participate in fertilization and zygote formation, the mutation will be inherited and transferred to all cells the offspring and a carrier or disease state might result according to the nature of the mutated gene and the pattern of its inheritance.

#### 7.4. Static and dynamic mutation

Static mutations signify mutations that are transmitted without change in type or extent of the mutation from a parent to his progeny. For instance, a father suffering from Marfan syndrome due to a specific mutation in the fibrillin gene may transfer the mutation as it is to some of his offspring. Mutation analysis by molecular diagnostic techniques of the gene of both father and child will reveal identity of the mutation between them in the affected gene. Static mutations characterize the mutational events that underlie the development of most currently defined genetic diseases.

Dynamic mutations, on the other hand, represent a peculiar category of genetic mutations that increase in magnitude or extent upon transmission from a carrier parent to an offspring. The discovery of this type of mutation clarified the pathogenetic mechanisms responsible for the pathogenesis of a large number of genetic diseases known as triplet repeat expansion disorders. The list of these diseases due to this type of mutation is expanding progressively and includes many common and relevant genetic disorders like fragile X syndrome, Friedreich ataxia, Huntington disease, myotonic dystrophy, some types of spino-cerebellar ataxia and many others.

In this type of mutation, a normal defined number of codons, triplets of nucleotides, along a specific segment of the gene increases in number when the gene is inherited. A threshold effect characterizes this dynamic mutational process. Within a certain range, we can identify a normal repeat number in the normal gene, a larger repeat number in carriers or pre-mutation state and a much larger number in affected patients. For instance, the fragile X mental retardation gene (FMR1 gene) which is mutated in fragile X syndrome, regulates the synthesis of a protein, fragile X mental retardation protein (FMRP) which acts, partly, as RNA binding protein that regulates in a global fashion cellular protein translation and microRNA regulatory pathways, and has important functions in many organs like the nervous system and the ovaries. The normal transcript of the gene has a sequence consisting of six to forty-five (6–45) repeats of CGG nucleotides at the 5'-untranslated region. Carrier mothers have a larger number of these repeats ranging from 55–200 repeats. When this pre-mutation state of the gene is transmitted to a male offspring, progressive increase, or dynamic expansion of the number of the repeats happens and the gene in the affected male offspring, or full mutation state, is found to contain more than 200, sometimes much more numbers up to hundreds, of these

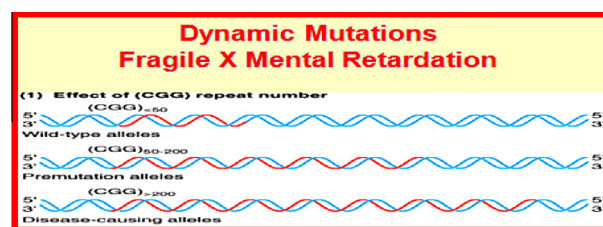


Figure 3 Triplet repeat expansion in fragile X syndrome.

repeats. The deleterious effects of this mutation and the resulting pathological phenotypes of the disease are due to disruption of functions of the FMR1 gene via silencing of the gene activity and cessation of synthesis of the FMRP, or through disrupting the structure of the gene leading to production of a defective protein that can't mediate its global cellular physiological regulatory functions in post-translation modifications and targeting of synthesized proteins (Fig. 3).

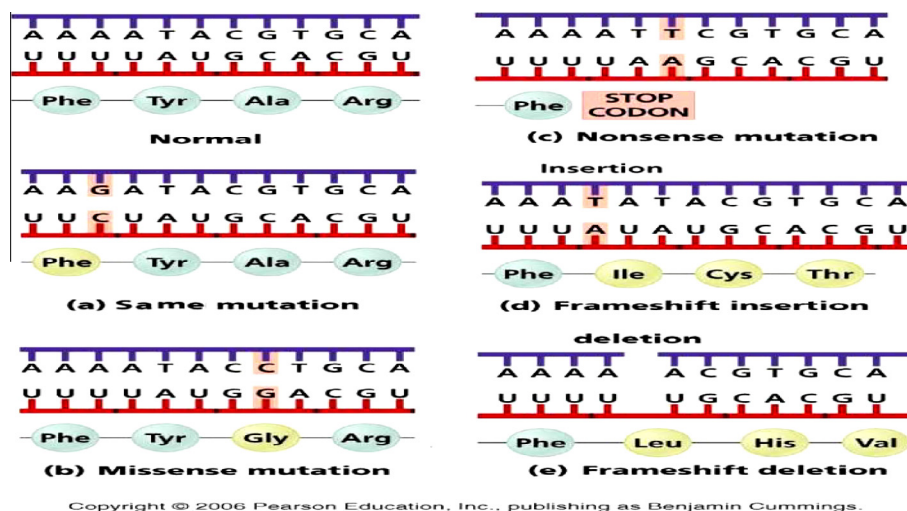
#### 7.5. Pathological and non-pathological mutation

The deleterious effects of mutation are determined by many factors. Mutations affecting functional elements of the genome, i.e. functional genes, can result in gene dysfunction causing deficient synthesis of the gene product or synthesis of defective product. These mutations are expected to cause pathophysiological changes and disease phenotypes in affected individuals, and represent an overt example of harmful pathological disease causing mutations. On the other hand, mutations affecting non-functional regions of the genome, e.g. intergenic areas of DNA and intronic segments of genes, do not result in pathophysiological alterations and are referred to as non-pathological mutations.

Though the vast majority of mutational events of the genome are harmful, some mutations, conversely, have beneficial effects. Such mutations, known as beneficial mutations, exert their effects by conferring selective advantage on affected cells secondary to the resulting change of the structure and function of protein/enzyme product of the mutated gene. The most obvious example of beneficial mutations is the sickle mutation of the  $\beta$ -globin gene. Carriers, but not homozygotes, of this mutation have a selective value because they are more resistant to infestation by the malaria parasite compared to normal subjects. Another example of beneficial mutations is the deletion mutation of the human CCR5 or CD195 or chemokine receptor type 5. The protein of the normal gene acts as a T cell membrane receptor and is used by human immunodeficiency virus (HIV) as a gate to enter the cell and build up infection with the virus. Mutation of the CCR5 gene, CCR5 Delta32 deletion, results in the synthesis of an altered protein that loses its receptor function and can't bind the HIV. Carriers of this beneficial mutation are resistant to infection and genetic homozygosity of the mutation is known to confer protection against infection with the virus [3].

#### 7.6. Point, small, gross and genomic mutation

Mutations are arbitrarily classified according to the size of affected component or region of the genome and the magnitude of the mutational damage into point, small, gross and



**Figure 4** Types & molecular mechanisms of point mutation.

genomic mutations. Point mutations refer to mutation of one single base of the gene irrespective of the size of the gene. Small mutations involve larger mutations of many bases, one or more exons or introns and one or more genes. Gross mutations comprise chromosomal abnormalities where tens, or even hundreds, of genes are affected by deletion/inversion/translocation of even tiny pieces of affected chromosomes. Genomic mutations represent the extreme end of the spectrum of mutations where the whole genome undergoes mutational changes. Triploidy and tetraploidy represent rare examples of structural or numerical genomic mutations. Also, aberrant development of vesicular moles and dermoid cysts represents clear examples of functional genomic mutations due to disordered imprinting of the whole haploid genome of the germinal cells following fertilization.

#### 7.6.1. Molecular mechanisms of point mutation

Point mutations refer to mutational events that involve one single base of the gene irrespective of the size of the gene. Lethal genetic diseases can be caused by single point mutations, even in very large genes. A well known example is Duchenne myopathy due to single point mutations of the dystrophin gene which is the largest human gene composed of 2.4 million bases. Sickle cell anemia is another example of a drastic genetic disease resulting from a single point mutation of the beta globin gene. Point mutations account for nearly 70% of currently defined human single gene disorders.

Molecular mechanisms of point mutation comprise a wide spectrum of alterations at the molecular level of the genetic material (Fig. 4). These mechanisms include replacement of one base (nucleotide) of the gene by another base, deletion, or loss, of one base of the gene and addition of one base to the gene. Replacement of one base by another base may not affect the function of the gene or, on the other hand, can have drastic effects on gene function according to the type and functional consequences of the change.

#### 7.6.2. Types of point mutations

Five different types of point mutation can be delineated: same-sense, missense, non-sense, re-sense and frame shift point mutations.

**7.6.2.1. Same-sense mutation.** Due to degeneracy of the genetic code that allows for defining the same amino acid by different codons, a point mutation might involve one base of a specific codon changing it to another codon, that still defines the same amino acid. For example, the amino acid leucine is defined or dictated by six different codons, TTA–TTG–CTT–CTC–CTA–CTG. Replacement of one base of some of these codons, TTA to TTG, CTT to CTC or CTA to CTG, by another base does not change the specified amino acid, leucine, in the synthesized protein, hence the term same-sense because the same amino acid is dictated by the new codon and no change in the synthesized protein results.

**7.6.2.2. Mis-sense mutation.** Due to the rigid rules of the genetic code, a change of one base of a codon might turn it into another codon that defines a different amino acid in the synthesized protein. If base replacement results in a new codon that specifies a different amino acid, a mis-sense mutation results. For instance, a point mutation of the codon (TTT) or (thymine–thymine–thymine) that defines phenylalanine may change it to another codon (TCT) or (thymine–cytosine–thymine) that defines serine. For protein translation system this change is considered as wrong code, hence the term missense, since it dictates a different amino acid. The effects of mis-sense mutation depend on the role played by the original amino acid in protein functioning, e.g. protein stability and aggregation. If it has important roles within fundamental structural or functional domains of the protein that could not be performed by the new different amino acid, then pathophysiological alteration of the structure and/or function of the protein are expected and the pathogenesis of a genetic defect might ensue.

**7.6.2.3. Non-sense mutation.** The sixty-four codons of the genetic code include three codons that do not define or dictate any amino acids and are used by the gene to signal the end of the protein synthesis process in order to form the proper protein with the proper number of amino acids. These codons (TAA–TGA–TAG) are termed stop or termination codons because they are recognized by both the mRNA transcript and the translation system as signals or markers of the end of the translation process since they do not define any amino



acids, hence the term non-sense, so that no more amino acids are to be added to the growing polypeptide chain.

Non-sense mutations result when a functional codon defining a specific amino acid is changed to a stop or termination codon. If base replacement results in the formation of a new stop or termination codon, for instance change of cytosine in (TAC) codon that defines tyrosine to adenine turns it to the stop codon (TAA) resulting in premature cessation of translation and the production of a short or truncated protein. The resulting alterations depend on many factors. If the mutation happens at the beginning of the coding part of the gene, most of the protein coded by the gene will not be translated from this point on. A short, or truncated, mostly non-functional protein will be synthesized and marked deterioration of gene function might result. If the mutation happens at or near the end of the coding part of the gene, most of the protein coded by the gene will be translated and less deterioration, or even no change, of gene function occurs. In both situations, the resulting pathogenetic alterations depend on the physiological role played by the missing non-translated part or domain of the protein. If these roles are important in mediating functions of vital metabolic networks in the cell, then genetic defects will happen and the pathogenesis of a genetic disorder might ensue.

**7.6.2.4. Re-sense mutation.** In this type of arbitrarily defined mutational event, the termination or stop codon at the end of the last exon of the gene might suffer a point mutation turning it to a functional codon. In this case, part of the un-translated region of the mRNA transcript will be included within the translation process which will continue with addition of more amino acids, not originally comprised in the protein sequence, till another stop codon is met with and recognition of the end signal and cessation of translation process occurs. The resulting abnormal pathological lengthening of the polypeptide chain results in the synthesis of a longer and larger protein. Such abnormal polypeptides are usually unstable macromolecules that degrade easily or faster than normal correspondent chains because of defective folding and improper structural configuration thus leading to quantitative functional deficiency. Also, due to their redundancy and defective primary structure, they might fail to undergo necessary post-translation modifications or post-translation targeting and trafficking to their proper cellular location. In either condition, genetic disease due to the functional deficiency of the protein might result.

**7.6.2.5. Frame-shift mutation.** Frame shifting signifies change of the specific sequence of the nucleotides of the gene due to deletion or addition of nucleotide(s) followed by corresponding change of the amino acid sequence of the synthesized protein. Deletion or addition of one base within the gene will result in shifting of the reading frame of the newly formed codons. This type of point mutation is called frame-shift mutation. The resulting effects may lead to the formation of new same-sense, missense or stop termination codons with ultimate consequences similar to the results of the similar aforementioned mechanisms. These same rules apply for mutational events involving the addition or deletion of two bases. If three bases are added or deleted, a whole new codon will be added or removed, respectively. Whereas addition or deletion of one codon to a functional exon of the gene might result in a wide spectrum of alterations as previously explained, whole codon addition or deletion within non-coding parts of the gene, introns, has no effect on translated protein or on gene function. Frame shifting of the gene sequence leads to frame shifting in the amino acid sequence of the synthesized protein and a new protein with new sequence of amino acids might be translated. The similarity between the amino acid sequence of the wild type protein and that of the new protein depends on the start site of the frame shift mutation of the gene sequence. These same rules apply for mutational events involving the addition or deletion of two bases. If three new bases are inserted between existing codons, however, a whole new codon will be added and an additional amino acid will be added to the polypeptide chain of the protein. Also, if three bases constituting a functional codon are removed or deleted from the gene, the amino acid specified by the deleted codon will not be translated and will be missing from the synthesized polypeptide chain. Whereas addition or deletion of one codon to a functional exon of the gene might result in a wide spectrum of alterations as previously explained, whole codon addition or deletion within non-coding parts of the gene, e.g. introns, usually has no effect on translated protein or on gene function [4].

Change in the structure of one base of the gene, e.g. methylation of cytosine, can affect gene transcription through several different mechanisms. Each cell has a specific methylation pattern necessary for normal cell differentiation during development. Changes in this pattern by mutations of regulatory genes controlling its timing and its magnitude can have marked deteriorating effects on the cell during differentiation. This type of mutation must not be confused with epigenetic

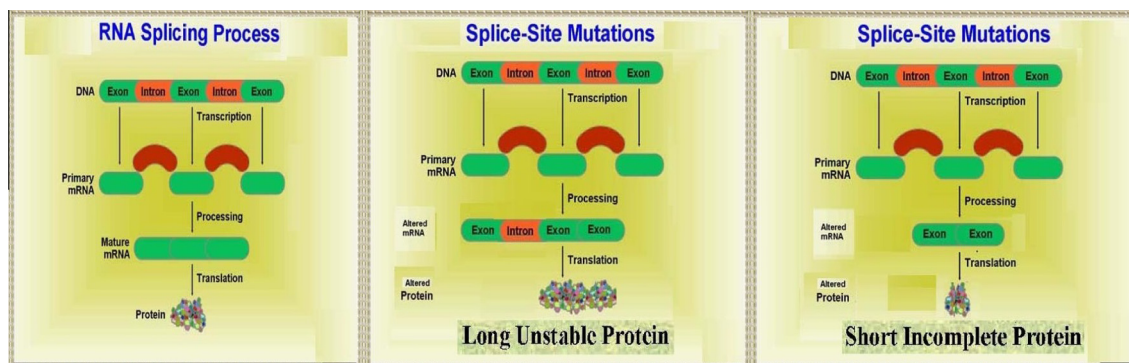
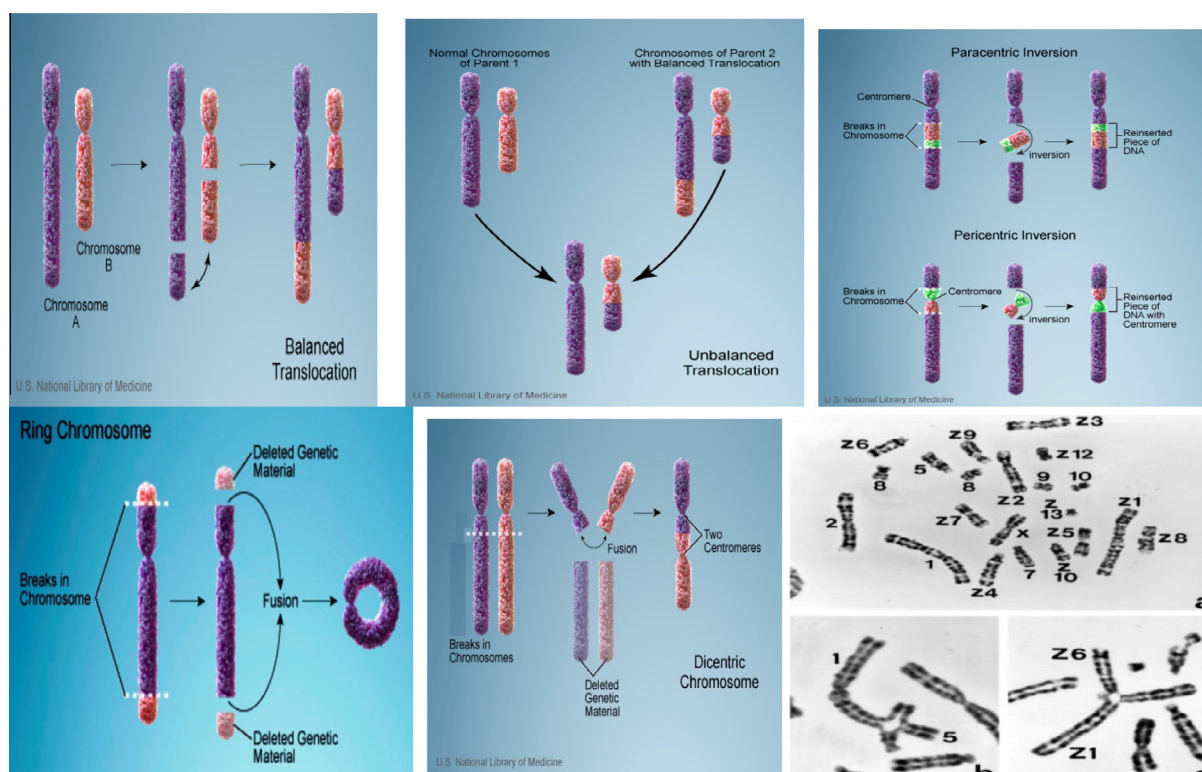


Figure 5 Splice site mutations.



CHROMOSOMAL MUTATIONS	
Structural Mutations	Numerical Mutations
1. Deletion	1. Trisomy (47 Chromosomes)
2. Translocation	2. Monosomy (45 Chromosomes)
3. Insertion	3. Hypodiploidy (Less than 46)
4. Ring chromosome formation	4. Hyperdiploidy (More than 46)
5. Dicentric chromosome formation	5. Triploidy (3N : 69 Chromosomes)
6. Chromosome gaps and breaks	6. Tetraploidy (4N : 92 Chromosomes)

**Figure 6** Types of chromosomal abnormalities.



**Figure 7** Structural chromosomal abnormalities.

mutations that involve structural changes in the chromatin or the DNA-associated proteins rather than the DNA itself [5].

### 7.6.3. Small mutations

Small mutations are arbitrarily classified so as to comprise structural changes involving more than one base of a gene. Accordingly, they span a very wide spectrum of pathogenetic structural alterations of the genetic material beginning with mutations involving at least two nucleotides and ending with mutations involving part of a gene, a whole gene or, even, few genes. Small mutations include addition or removal of two or more bases, addition or removal of one or more codons, single or multiple exon deletion or duplication, single

or multiple intron deletion or duplication, deletion or duplication of a gene or of few genes, amplification of genes, inactivation of genes and transposon-induced mutations.

The pathophysiological consequences of these mutations depend on the resulting changes in either the genetic regulatory networks or the metabolic networks of the cell secondary to deficient and/or defective synthesis of the gene product. The resulting deterioration of gene function leading to the pathogenesis of a genetic disorder correlates with the extent of deficiency of the gene product, whether due to actual deficiency of the protein or regulatory small RNA coded by the gene due to suppression of gene function and inability to synthesize the gene product, or to relative or absolute deficiency of the

function of a faulty synthesized gene product. Deletion of large portions of a gene, multiple exon deletions for instance, result in the synthesis of grossly defective protein lacking most or much of its domains needed to mediate its destined physiological roles causing marked pathophysiological deteriorations in the functioning metabolic networks dependent on these roles, thus predisposing to development of genetic disorders.

Small mutations of bases involved in splicing mechanisms mediating intron excision and splicing of remaining exons, as part of the post-transcription modifications of mRNA, can have marked effects on the translation efficiency of the final mRNA and is a well-known mutational mechanism that underlies the pathogenesis of many common and serious genetic diseases (Fig. 5). These splice site mutations may result in over excision of parts of adjacent exons and synthesis of shorter polypeptide chains. If the missing amino acids coded by the over excised parts are important for mediating protein function or maintaining its structural integrity, then pathophysiological alterations and pathogenesis of disease might be expected. On the other hand, if splice site mutations result in skipping of an intron, part of an intron, a whole intron or multiple introns from excision, the resulting translated protein might be large enough to be unstable and easily degradable, or its structural configuration might be altered in such a way that its functional domains are no more accessible to each other or to other components, e.g. substrates or intermediary metabolites, needed for mediating its physiological functions, and a pathogenetic defect results.

#### 7.6.4. Gross mutations

Gross mutations are mutations that involve large portions of the genetic material. Chromosomal aberrations represent the prototype of this type of mutation, since even the smallest functional portion of a chromosome may consist of tens to hundreds of genes. Due to the peculiar structural organization of the nuclear human genome, chromosomal abnormalities can involve either the structure of the chromosome, structural chromosomal abnormalities or the number of the chromosomes, numerical chromosomal abnormalities. Mutations of chromosome structure might involve the autosomes, autosomal chromosomal abnormalities, of chromosomes 1–22, or sex chromosomal abnormalities involving the sex chromosomes (X and Y chromosomes). Structural chromosomal aberrations include different pathogenetic types like deletions, duplications, inversions, ring chromosome formation, unidirectional translocation, mutual translocations and Robertsonian translocations, chromosome gaps and breaks, and other less common abnormal types. Numerical chromosomal abnormalities that affect the number of chromosome encompass varied group of aberrations including trisomy, monosomy, hypodiploidy, hyperdiploidy, triploidy and tetraploidy (Fig. 6) [6].

##### 7.6.4.1. Types and molecular mechanisms of chromosomal aberrations. (1) Structural chromosomal aberrations (Fig. 7).

###### (a) Deletion

Chromosome deletion means loss of part of the chromosome. It may be terminal deletion when it involves the end piece of the chromosome or interstitial deletion when it affects other parts of the chromosome. Interstitial deletions involving the centromeric region of the chromosome lead to formation

of acentric chromosomes. Because chromosome segregation during cell division depends on attachment of the spindle fibers to specific centromeric receptor proteins, acentric chromosomes are lost during the anaphase stage of cell division and chromosome monosomy of affected daughter cell results. Autosomal monosomy is incompatible with life of the cell in view of the deleterious functional deficiency of the proteome due to loss of hundreds, even thousands, of genes located on the missing chromosome.

###### (b) Translocation

Translocation involves breakage of part of the chromosome and its insertion within the genetic material of another chromosome. It may be unidirectional when a segment of the chromosome is broken at both ends, detached from its original site and translocated to another chromosome, or it may be reciprocal when two different chromosomes interchange parts of their genetic material. Translocation can result in functional deficiency and development of genetic disease if the translocated chromosome segments are inserted within intact gene sequences on the recipient chromosome leading to its disruption. Conversely, translocation might lead to over expression of the genes at the end of the translocated segment if they get inserted next to an over expressive promoter of a recipient gene. Translocations leading to over activation of oncogenes or causing disruption of critical regulatory genes, or genes responsible for maintaining genomic stability and integrity, are known to underlie the development of many malignant tumors secondary to these peculiar pathogenetic mechanisms. Examples of such cancers include Burkitt lymphoma  $t(8;14)$ , follicular carcinoma of the thyroid gland  $t(2;3)$ , Ewing sarcoma  $t(11;22)$ , chronic myelogenous leukemia  $t(9;22)$ , follicular lymphoma  $t(14;18)$  and many others.

Robertsonian translocation is a specific type of translocation involving translocation of a small (21–22) or large (13–15) acrocentric chromosome to another, large or small acrocentric chromosome. This defect involves fusion of the long arms of the two acrocentric chromosomes at their centromeres and loss of both short arms of the two chromosomes. Balanced carriers of this defect are phenotypically normal because they do not suffer relevant genetic deletions, since the short arms of acrocentric chromosomes do not code for essential or unique gene products.

Robertsonian translocations have an incidence of about one in a thousand newborns with its most frequent forms being between chromosomes 13 and 14, 14 and 21, and 14 and 15. Most people with Robertsonian translocations have only 45 chromosomes in each of their cells. Their children, however, might inherit the fusion chromosome and become phenotypically normal balanced carriers like their carrier parent or they may inherit a missing or an extra long arm of an acrocentric chromosome. In unbalanced forms, Robertsonian translocations cause chromosomal deletions or addition and result in chromosome trisomy syndromes including trisomy 13 (Patau syndrome) and trisomy 21 (Down syndrome). Rarely, if both parents are heterozygous for the same translocation, they have the possibility of giving birth to homozygous viable offspring with 44 chromosomes in each of their somatic cells.

###### (c) Inversion

Inversion denotes breakage of a chromosome segment at both ends and its reinsertion at its original site in reversed up-down position. Inversions are classified into two main types depending on whether they include the centromere, pericentric

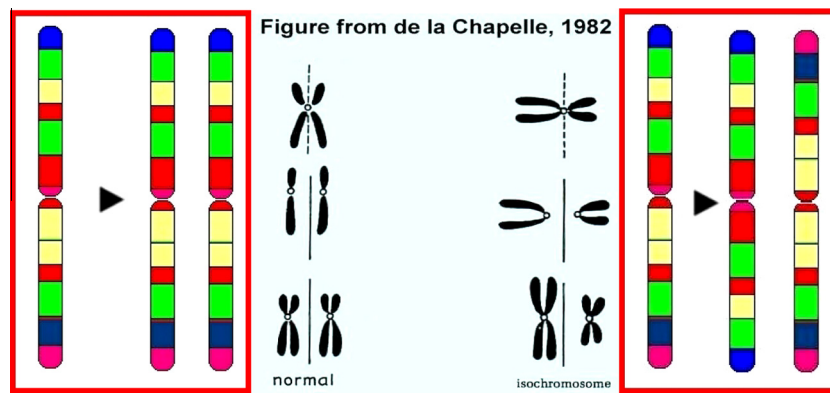


Figure 8 Isochromosome formation.

inversions, or occur on one arm of the chromosome away from the centromere, paracentric inversions. Since inversions do not result in loss or gain of extra genetic material, inversions do not cause marked functional deficiency. However, if the break points of the inversion disrupt the sequence of an important gene, disease state might ensue. Inversions result in disturbed crossing over during meiosis and production of defective gametes with low recombination frequency. Depending on the extent of chromosome segments affected by the inversion, these defects might be incompatible with normal development and lead to early spontaneous abortions.

#### (d) Ring chromosome formation

Ring chromosomes are incomplete chromosome segments resulting from terminal deletion of variable parts of both chromosome arms with subsequent joining of both arms to form ring structures. The net loss of genetic material from the chromosome depends on the extent of deletion of both arms. Ring chromosome formation can also occur with terminal or total deletion of one chromosome arm only, and in rare cases it occurs when telomeres at the ends of a chromosome fuse without any loss of genetic material from the chromosome. Pathological consequences of ring chromosomes are caused by deletion of genes in the telomeric regions of affected chromosomes.

#### (e) Duplication

Chromosome duplication occurs when part of a chromosome is copied abnormally, resulting in extra genetic material from the duplicated segment. Duplications arise from aberrant recombination or unequal crossing-over that occurs between misaligned homologous chromosomes during meiosis with consequent reciprocal deletion of the homologous chromosomes. Chromosome duplications offer the etiological basis of gene duplication and gene amplification. They also offer an explanation for the presence of multiple repetitive sequences along the DNA, since placement of the duplicated regions adjacent to the original sequence results in formation of tandem repeats along the DNA. Gene amplification due to duplication is a common phenomenon for many oncogenes because duplication and amplification of oncogenes results in their over expression with over production of oncoproteins that confer the malignant phenotype on transformed cells. Examples of human cancers due to oncogene duplication and amplification include esophageal cancer, gastric cancer, cervical cancer, colorectal cancer, breast cancer and neuroblastoma.

The finding that specific genomic regulatory elements, e.g. zinc finger nuclease enzyme, can induce specific chromosomal rearrangements, like duplications and inversions, suggest an evolutionary role for these chromosomal abnormalities. This event might be considered as a mechanism of genomic evolution through formation of novel genes that can amplify the function of parent genes or, alternatively, can undergo one or successive mutational events converting them to new genes with new functions. However, the beneficial role of this presumed evolutionary pathway as identified in some organisms, e.g. the mutational conversion of a duplicated digestive gene in ice fish into an antifreeze gene [7] is still a matter of debate waiting for clarification in human race since most of these chromosomal rearrangements have pathological effects on affected subjects. This might be attributed to their being an expression of genomic instability, rather than of genomic evolution, of the human genome.

#### (f) Isochromosomes

Normally, duplicated chromosome pairs separate from each other and get equally distributed to daughter cells by the attachment of the spindle fibrils to specific receptor proteins on their common centromere with consequent disjunction along their longitudinal axis (Fig. 8). Abnormal attachment of the spindle fibrils or instability of the centromeric region might result in abnormal separation of the chromosome pair along its transverse axis so that two chromosomes result: one consisting of the long arms of the pair and the other consisting of the short arms only, i.e. mirror-image chromosomes. This results in complete deletion and functional monosomy of the short arm or the long arm of each resulting isochromosome, respectively. Isochromosomes have equal arms with identical banding pattern of both arms. They are seen in some female patients with Turner syndrome and in many types of myeloid and lymphoblastic leukemias. They may rarely involve autosomes, e.g. isochromosome 18q syndrome.

#### (g) Mosaicism

Chromosomal mosaicism describes the presence of two or more cell populations with different chromosome complements in one individual. For instance, mosaic Down syndrome (46,XY/47,XY,+21), mosaic Turner syndrome (46,XX/45,X) and mosaic Klinefelter syndrome (46,XY/47,XXY). Normally, due to Lyonization or X chromosome inactivation, all female cells are functionally mosaic as regards the X chromosome, i.e. cells with the maternal X inactivated depend on the paternal X chromosome genes for mediating X



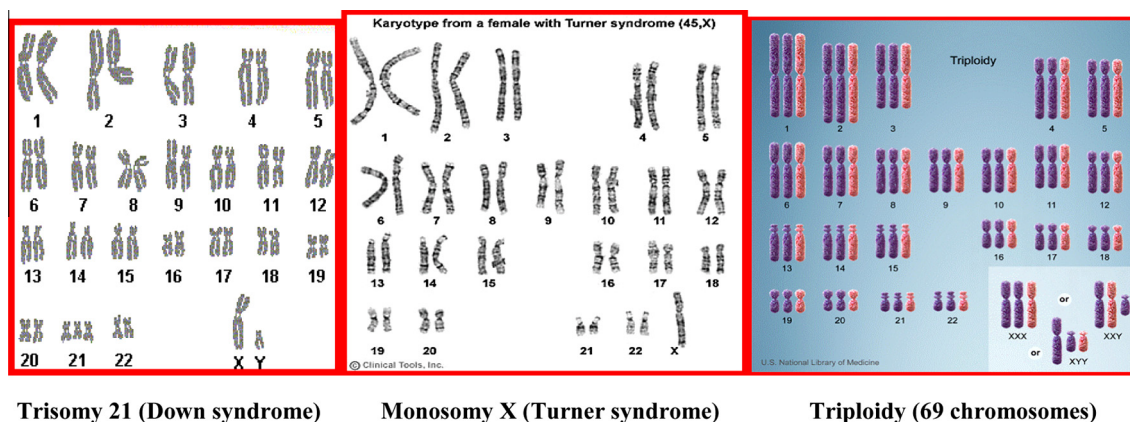


Figure 9 Numerical chromosomal abnormalities.

chromosome-dependent genetic functions, and vice versa. Pathological mosaicism, on the other hand, occurs when a mutational event selectively affects one cell and gets transmitted to all descendent daughter cells. It can affect gonadal cells during meiosis resulting in some gametes carrying the mutation with the rest of the germ cells being free from mutation, gonadal mosaicism, as well as somatic cells at any stage along the pathway of cell division after the first post-zygotic division.

Accumulating evidence suggests that chromosomal mosaicism, probably, represents a significant genetic phenomenon with widespread effects on many aspects of human biology and health since a significant proportion of human pathogenic conditions is associated with chromosomal mosaicism. Though mosaicism is a clear demonstration of genetic diversity, its true significance remains, as yet, unknown in view of the contradictory genetic effects attributed to and associated with its occurrence. For instance, mosaicism is quite a frequent finding among human fetuses and is observed in nearly 25% of spontaneous abortions. It also plays a role in the generation of meiotic aneuploidy known to be the leading genetic cause of human prenatal death, congenital malformations, prenatal mortality and postnatal morbidity, many neuropsychiatric disorders, chromosomal syndromes, learning disabilities, cancer and immune diseases. Contrariwise, there is strong and substantial evidence suggesting that increased incidence of mosaic aneuploidy in the developing human brain is an integral component of normal prenatal development of the human central nervous system.

#### (h) Chromosome gaps and breaks

These distinctive types of chromosomal abnormalities reflect effects and consequences of mutations leading to interruption of the integrity of the chromosome DNA. They, probably, predispose to development of many other types of structural aberrations of the chromosomes [8].

#### (2) Numerical chromosomal aberrations

Numerical chromosomal abnormalities refer to chromosomal abnormalities caused by the presence of abnormal number of chromosomes within the cell. These gross mutational events can involve autosomes, e.g. trisomy 21 in Down syndrome and hypodiploidy and hyperdiploidy in cancer cells, or the sex chromosomes like monosomy X (45,X) in Turner syndrome and XXY abnormality in Klinefelter syndrome (47,XXY) (Fig. 9).

(a) Trisomy, or the presence of three copies of a chromosome instead of two, results from non-disjunction, or non-separation, of duplicated chromosomes after replication. Normally, during cell division, replication of DNA occurs and chromosomes duplicate themselves so that each daughter cell receives the same number of chromosomes like the parent cell, in order to keep genetic identity of the species. If a duplicated chromosome pair fails to separate from each other and both chromosomes are distributed to one daughter cell only, then trisomy of this cell results with concomitant monosomy of the other daughter cell. Partial trisomy refers to duplication of certain parts of a chromosome, e.g. the long arm or the short arm only. It has similar etiological bases like complete trisomy including non-disjunction, spindle derangements and centromeric breaks due to genomic instability.

(b) Monosomy means the presence of one single copy of the chromosome. Autosomal monosomy is incompatible with normal development and cells with this type of abnormalities die because of lack of the vital functions of hundreds or thousands of genes on the missing chromosome. Monosomy of the X chromosome is the only monosomy compatible with life in view of the peculiar nature of the genetics of the X chromosome. Partial monosomy, due to deletion of a part of a chromosome, is a common cytogenetic abnormality seen in many genetic syndromes. The spectrum of pathological findings in each syndrome depends on the extent of functional impairment caused by lack of functions of genes lost due to the deletion.

(c) Hypodiploidy refers to chromosome complement less than the normal diploid (46) chromosome number of somatic cells. Cells of Turner syndrome with 45 chromosomes represent the only viable hypodiploid events. Hypodiploidy is a common cytogenetic abnormality in many malignant cells. In some cases it might be so extensive that some malignant cells have chromosome number reaching near haploidy, or 23 chromosomes. Hypodiploidy is an important prognostic marker for the karyotypic evolution of cancer cells since the smaller the number of chromosomes a cell can live with, the more virulent malignant phenotype these cells can have. Milder states of hypodiploidy, low hypodiploidy with (33–38) chromosomes and high hypodiploidy with (42–45) are also common cytogenetic findings in malignant cells.

(d) Hyperdiploidy, or chromosome numbers more than 46 chromosomes, underlies the pathogenesis of some genetic



disorders, e.g. trisomy 13–18–21, XXY, XYY and multiple X syndromes with three or more X chromosomes in cells of affected female patients. Marked pathological hyperdiploidy with chromosome number reaching up to seventy (70) or a little bit more chromosomes, however, is quite common in many malignancies like acute lymphoblastic leukemia and acute myeloid leukemia [9].

#### 7.6.4.2. Pathogenetic mechanisms of chromosomal aberrations.

The pathogenetic mechanisms involved in the pathogenesis of chromosomal aberrations are, still, mysterious and hardly understandable. They cannot be interpreted on molecular basis even for chromosome gaps and breaks in view of the complex structural assembly of the chromosome and its intimate integrity with the chromatin material. Gross regulatory defects of mechanisms controlling cell division, including the formation of the spindle and the timing of its action in synchronization with other biochemical and signal transduction effectors are hold responsible for predisposing to non-disjunction and the development of chromosomal trisomies and chromosomal monosomies as well. Similar defective regulatory mechanisms might underlie the development of polyploidy conditions like triploidy and tetraploidy.

However, the regular and persistent occurrence at nearly constant incidence rates of well recognized and well defined genetic defects, including chromosomal aberrations, irrespective of ethnic, racial or environmental factors, indicates that these types of genetic changes or mutations might have, still unknown, deep rooted significance background in our genome. Disclosure of master genes and related genetic factors responsible for regulating both the stability and behavior of the genome as a whole will, surely, throw more light on factors underlying pathogenesis and development of chromosomal abnormalities.

#### 7.6.5. Genomic mutations

Genomic mutations refer to mutations involving the whole genome (either the 23 chromosomes haploid genome or the 46 chromosomes diploid genome). They comprise numerical genomic mutations involving the whole chromosome set of the genome like triploidy (69 chromosomes genome) and tetraploidy (92 chromosomes genome). They, also, include functional genomic mutations leading to disturbed and/or defective expression of the whole genome functions. Genomic functional mutations reveal their consequences in many phenotypes. In humans, the development of vesicular or hydatidiform molar pregnancies and the formation of dermoid cysts, instead of normal development of the zygote, represent obvious examples of genomic functional mutations. Normally, during the first five days following fertilization and zygote formation, all cellular processes including cell growth, differentiation and division are controlled and mediated exclusively by the maternal genome of the ovum. The sperm genome begins its participation in these processes after these critical five days post-fertilization period. Improper temporal programming of these genomic balances and interactions, e.g. due to genomic imprinting mutations, leads to disturbed genomic regulation of development and differentiation of the developing zygote, with drastic consequences leading to actual cessation and disturbed progress of normal development and differentiation. If the maternal genome fails to start functioning properly after fertilization or fails to maintain its genetic regulation of development over the critical immediate post-fertilization period, or

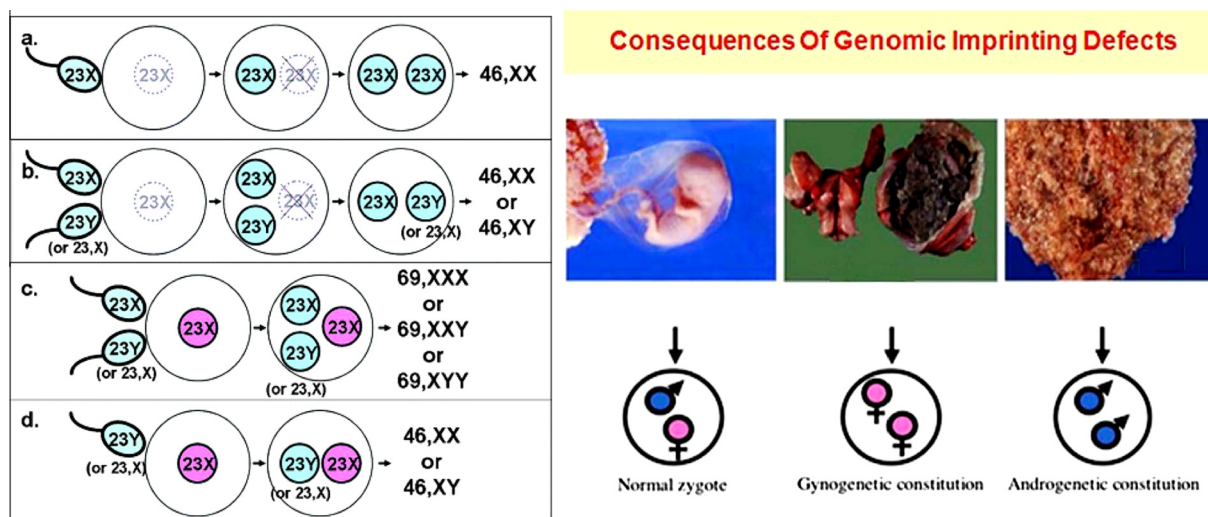
if the paternal genome is prematurely induced to start its roles in genetic regulation of development and differentiation as a result of these genomic imprinting mutations, normal embryogenesis and/or fetal growth is brought to a stand-still [5].

Genomic functional mutations might, also, be caused by other pathogenetic mechanisms, one of these abnormal mechanisms entails exclusive predominance of one parental genome in zygote formation and development. The diploid genome of the zygote may be formed from two maternal haploid genomes (46, XX) without any sperm genome, or from two paternal haploid sperm genomes (46, YY) without participation of any maternal genome. These abnormalities in chromosomal constitution of the zygote can be caused by many pathogenetic mutational events. Endoreduplication of one parental genome of the zygote with suppression, involution and disappearance of the other genome, or fertilization of the ovum by two sperm genomes with consequent disappearance of the ovum genome from the developing zygote can lead to pathogenesis of these genomic chromosomal abnormalities. A well-known wide spectrum of pathological embryonic and fetal malformations and abnormal conception products results from these genomic functional mutations (Fig. 10). [6].

Normal development of human embryos is an extremely complex process mediated under strict control of thousands of regulatory genomic pathways. Fertilization results in bringing up two different genomes that must work together in precise concert to attain the final aim of the whole process, that is formation of a normal complete offspring. Though the immediate post-fertilization period is the most genetically active among all other periods of life of the organism, selective activation and silencing of the large number of genes constituting the genome of the zygote is a remarkable feature of this early stage. Some maternal genes are activated and others are silenced. The same process applies to parental genes as well. This phenomenon of selective activation or silencing of genes based on their parental origin is referred to as imprinting. Imprinting might involve a single gene, multiple genes or most genes on a chromosome as in Lyonization of the X chromosome, where it is known as gene or genetic imprinting, or it might involve a whole set of chromosomes of a certain parent, a condition referred to as genomic imprinting.

Imprinting seems to be a global biological feature of all living systems. In spite of accumulating observations, it remains to be one of the most puzzling phenomena in biology because of lack of sufficient information regarding the underlying mechanisms that mediate its effects and also due to the many species-specific and inter-species unrelated aspects and mechanisms of imprinting. For instance, regulation of expression of specific genes by imprinting is unique to therian mammals, i.e. placental mammals and marsupials, and flowering plants. The majority of imprinted genes in mammals have fundamental roles in embryogenesis and development of the placenta. Other imprinted mammalian genes are involved in post-natal developmental processes. Within a different biological context, for example in insects, imprinting plays a crucial role in sex determination where genomic imprinting of the whole paternal genome with consequent functional haploidy is a prerequisite for normal development into males.

Though many hypotheses have been proposed to explain the biological aims and the evolutionary origins of imprinting, e.g. natural selection hypothesis and parental conflict hypothesis, imprinting in essence represents a programmed regulatory



**Figure 10** pathological consequences of genomic structural and functional mutations.

mechanism of the genome to harmonize functions and interactions of the large number of genes that constitute it, so that integrity and stability of the genome, hence genomic identity, is maintained, preserved and conserved in spite of the continuously ever changing proteome phenotype and proteome dynamics of living systems. If genes constituting a specific genome are imprinted, either expressed or silenced, without strict regulatory mechanism(s) controlling temporal aspects of their functions, widespread random and conflicting qualitative and quantitative changes of the proteome synthesized by that genome will disturb the stability and integrity of the genome, through many different mechanisms, leading ultimately to decadence and loss of the genomic identity that characterizes living organisms [10].

#### 7.7. Base, sugar, phosphate mutation

Mutations, in general, refer to changes of the bases of the nucleic acids (A,G,C,T,U) and represent the commonest types of mutations of the genetic material. However, several kinds of mutations can affect the sugar and the phosphate group, and can have deleterious effects on the genetic material. Several kinds of mutation can affect the sugar portion of the nucleotide. For instance, some physical and chemical mutagens can add oxygen to the deoxyribose of DNA or remove oxygen from the ribose of RNA, or a whole deoxyribonucleotide might be substituted by a ribonucleotide. Such mutations of DNA, containing a ribonucleotide instead of a deoxyribonucleotide are usually silent, but under abnormal conditions they might be attacked by ribonucleases leading to DNA breaks. Another type of mutation of the sugar portion of the nucleotide involves methylation of ribose of a coding nucleotide in mRNA and can lead to silencing or, even, to total suppression of translation.

Mutations that affect the phosphorus atom of the nucleotide can cause widespread detrimental effects on the integrity of the DNA strand. Irradiation turns ordinary phosphorus to radioactive phosphorus which, upon release of an electron, changes to non-radioactive sulfur and suffers a recoil in diameter leading to loss of its connection to the adjacent sugar. This results in multiple recoil breaks in the sugar-phosphate

backbone of the DNA with ultimate breakage mutations of the nucleic acid [1].

#### 7.8. Persistent and reversible mutation

Mutations are frequent structural genetic alterations in view of the dynamic nature of the genome. It almost always accompanies replication of DNA and transcription of RNA. However, most of these mutations are corrected and repaired by the genomic repair mechanisms. Mutations in one cell that escape repair are liable to be transferred to daughter cells upon division and a whole progeny of mutated cells makes its appearance. Persistence of the mutation and its transfer to progeny cells results in fixation of the mutation, the change becomes persistent and appears in all daughter cells. The outcome of this scenario depends on the type of the cell and the pathogenetic effects of the resulting damage. Acquired persistent somatic mutations have the same effects as classic somatic mutations, they can cause cell death, dysfunction, apoptosis or malignant transformation. Acquired persistent germinal mutations might be transferred to offspring, become heritable and result in inherited genetic disease.

Since mutations are un-programed genetic events, the term reversible mutations is actually a misnaming since these mutations represent programed structural genetic alterations aiming at conducting specific purposes, e.g. regulation of gene function. For instance, methylation of bases to silence gene transcription, acetylation of regulatory DNA associated histones to enhance transcription and methylation of the adenine base in some types of mRNA/siRNA species to regulate energy balance or ATP homeostasis of the cell, are examples of such reversible changes of the genetic material. These alterations are sometimes considered as various mechanisms of epigenetic alterations, i.e. temporary imposed structural changes that do not alter the basic nucleotide sequence of DNA. For simplification and to obviate confusion, at least within the scope of human genetics, it might be more plausible to omit both terms, epigenetics and reversible mutations, and refer to structural genetic alterations either as mutations or as temporary structural genomic alterations.

## 8. Pathogenetic mechanisms of genetic diseases

The genetic material controls life activities of the cell through regulating synthesis of proteins which directly mediate these activities. Regulatory genes, in addition, control the transcription of many classes of small RNAs that have fundamental roles in direct and feed-back regulation of most aspects of the genetic material.

Mutations cause structural alterations of the genetic material. Depending on the site, nature, magnitude and effects of the mutational event as well as on the functions and importance of the mutated genes, pathogenetic mechanisms that result in deficient synthesis of gene products, synthesis of defective gene products or disturbed regulation of cellular activities will lead to development of genetic disorders, secondary to the ensuing pathophysiological alterations of cellular functions [9].

Maintaining stability, integrity and species-specific identity of the genome represents a prerequisite, not only for executing cell functions properly, but more fundamentally for beginning, continuation and conservation of life. It is the preservation of the collaborative and integrated intimate relationship of these three aspects of the genome that represent the real kernel and true essence of existence of all forms of life. Many pathogenetic mechanisms that drastically affect genome stability and genome integrity have been defined. These particular groups of mutations constitute important detrimental events that act via different pathways, e.g. defective genetic repair mechanisms or premature induction of apoptosis, leading ultimately to loss of genome stability and integrity and induction of cell death. On molecular or cellular level, these mutations might be considered as life-ending mutations. These mutations might also act in a different way through total loss of reproductive fitness and disappearance of individual genomes from the gene pool of certain species.

The spectrum of pathogenetic mechanisms and the resulting pathophysiological disturbances that underlie the development of genetic disorders is quite wide in view of the complexity of the structural organization of the genome and the strict functional specialization that characterizes each of its components. Additionally, the obscure nature and unclear functions of many components of the genetic material, undoubtedly, conceal many, still unknown, pathogenetic mechanisms and hinder proper understanding of their exact pathways. It is hoped that final completion of the human genome project might disclose the exact and complete structural organization of the human genome [11]. However, a parallel human genome function project aiming at defining the complete functional spectrum of the genome seems to be an indispensable and imperative task in order to finalize our knowledge of our genetic material.

Currently defined pathogenetic mechanisms and pathophysiological alterations implicated in the pathogenesis of genetic disorders include the following:

- (1) Loss/damage/duplication/inactivation of nuclear genes
- (2) Mutation of mitochondrial genes (mitDNA)
- (3) Deficient/defective DNA replication/repair
- (4) Triplet repeat expansion disorders
- (5) Loss/acquisition/damage of chromosomes
- (6) Deficient transcription of mRNA

- (7) Transcription of defective mRNA
- (8) Deficient/defective post-transcription mRNA repair
- (9) Deficient/defective post-transcription modifications of mRNA
- (10) Deficient translation of proteins
- (11) Translation of defective proteins
- (12) Deficient/defective post-translation modification of proteins
- (13) Deficient/defective post-translation repair of misfolded proteins
- (14) Deficient/defective post-translation targeting and trafficking of proteins
- (15) Deficient/defective regulation of cell growth
- (16) Deficient/defective regulation of cell division
- (17) Deficient/defective regulation of cell differentiation
- (18) Deficient/defective regulation of cell migration
- (19) Deficient/defective regulation of intercellular contact and cell movement
- (20) Deficient/defective apoptosis/selection repair
- (21) Deficient/defective regulation of cell architecture and cytoskeleton: e.g. ciliary dyskinesia disorders (bronchiectasis, dextrocardia and situs-inversus, hydronephrosis, hydrocephaly, male infertility and repeated abortions), hereditary spherocytosis, Wiskott–Aldrich syndrome and neural tube defects.
- (22) Imprinting disorders: genomic imprinting disorders, e.g. ovarian teratomas and hydatidiform moles, and genic imprinting diseases, e.g. Prader–Willi syndrome, Angelman syndrome, Silver–Russell syndrome, Beckwith–Wiedemann Syndrome.
- (23) Deficient/defective regulation of cellular functions:
  - (a) Deficient/defective transport across cell membrane or membranes of cell organelles (transport defects)
  - (b) Deficient/defective transport across cell pores, nuclear pores or pores of cell organelles (channelopathies)
  - (c) Deficient/defective secretion of gene products (protein/enzyme deficiency disorders)
  - (d) Deficient/defective excretion of metabolic waste products (storage disorders)
  - (e) Deficient/defective regulation of intra and inter network reactions and interactions: signal transduction disorders: e.g. neurodegeneration, diabetes mellitus, schizophrenia and Noonan syndrome.
  - (f) Deficient/defective positioning of structural proteins (cell cytoskeleton disorders)
  - (g) Deficient/defective regulation of intracellular trafficking.
  - (h) Deficient/defective production of cellular energy: oxidative-phosphorylation disorders.
  - (i) Ubiquitination/proteasome degradation defects: e.g. Friedreich ataxia, Huntington disease, Parkinson disease, Alzheimer disease, Angelman syndrome, motor neuron disease and immunodeficiency.
  - (j) Apoptosis defects: e.g. congenital malformations, autoimmune disorders, cancer and neurodegeneration.

## 9. Anti-mutation mechanisms of the human genome and human proteome

The human genome develops, persists and works in a hostile environment full of existing, and continuously generated,



mutagens. Mutational events induced by external factors, which include physical, chemical and biological mutagens, have widespread detrimental effects on the stability and integrity of the genome as well as on the stability and integrity of the proteome. Additionally, further and considerable damage of the structural organization and functional capabilities of both the genome and the proteome regularly occurs on continuous and progressive basis due to the continuously generated burden of internal mutagens that result from the diverse metabolic activities of the exceedingly large number of metabolic networks of the cell. Unless a powerful and effective protective and repair system actively participates in protecting the genome and proteome of the cell against the deleterious effects of mutations, and in efficient repair of resulting damage, maintaining the stability and integrity of both of these bio-systems that constitute the framework of life activities within the cell would have been impossible.

The human genome is endowed with a spectacular multifaceted strong anti-mutation system responsible for maintaining stability and integrity of the genome and preserving its identity. It acts by protecting the genome from the detrimental effects of mutation and by repairing mutation-induced damage. Obviously, the balance between the pathological effects of mutation and the ability of the anti-mutation system to counteract and to reduce the consequences of these effects represents the main factor that determines the likelihood of having a mutation-induced genetic disease. The human anti-mutation system comprises both innate mechanisms common to, and shared by, all individuals, e.g. degeneracy of the genetic code, and acquired aspects determined by the inherited genetic background of each human being, e.g. DNA repair system.

The human transcriptome, being subjected to the same mutational events that can affect, alter and damage the DNA, seems to have efficient anti-mutation mechanisms to guard against occurrence of errors during RNA transcription and to correct and repair some post-transcription defects of mRNA that can cause errors during protein translation. A separate RNA-proofreading system seems to exist and it probably acts during transcription by relying on the sequence complementarity information or database stored within the complementary silent or non-transcribing strand of DNA. Depending on the sequence of the active strand to ensure accurate transcription might result in improper transcription if mismatch errors occur due to, e.g. polymerase dysfunction. This assumption might, partly, explain the still un-understandable behavior of gene function which involves, seemingly needless, indirect and energy consuming mechanisms by transcribing a complementary mRNA molecule, rather than an identical mRNA, that has to be decoded again by rRNA and tRNA in the ribosome during translation.

## 10. Anti-mutation mechanisms of the human genome

### 10.1. Structural organization of the human genome

The peculiar structural organization of the human genome represents the first innate anti-mutation mechanism in view of the presence of large interspersed portions of non-functional intragenic, introns, and inter-genic DNA sequences and segments that can be mutated without having appreciable deleterious functional effects. In addition to functional sequences needed

for synthesis of protein and of regulatory small RNA species, the human genome has a considerable amount of repetitive DNA sequences, including both noncoding repetitive DNA sequences and multiple copy genes and gene fragments, a large number (19,000–21,000) of pseudogenes, a considerable sizable portion (about 1/6th of the total genome size) as pyknons, a quite large portion (nearly 40% of the total genome size) as transposons and large numbers of multiple copies of functional genes that share the same regulatory function and whose suppression or damage by mutation can be tolerated by other genes having the same function. These peculiar structural features of the human genome allows for occurrence of mutational events in many segments of the genome without having appreciable functional defects. Even if some of these genes or DNA sequences have important roles in genome function, their presence in multiple repetitive copies can greatly reduce, or even nullify, the consequences of mutational damage resulting from affecting many copies.

The presence of multiple copies, hundreds to thousands, of mitochondrial genes within the mitochondria of each cell is crucial in obviating devastating mutation-induced damage to these vital organelles in view of their role in production of ATP. This feature of mitochondrial genome allows for considerable burden of mutations to affect it before appreciable pathological consequences result. It is estimated that mutations affecting nearly 80% of certain mitochondrial genes might occur before pathological manifestations of mitochondrial genetic diseases make their appearance due to this multiple copy feature of mtDNA [1].

### 10.2. Structural features of DNA

DNA exists as a double stranded structure composed of two tightly bound strands, each strand consisting of a straight sugar-phosphate backbone with opposing nitrogenous bases linked by glycosidic linkage to the sugar of one strand and by hydrogen bonds with a complementary base on the other strand. This specific structural organization of DNA serves many purposes. It stabilizes the dynamics of the molecule, permits replication and duplication of the genetic material, protects the interiorly located bases and, most important, stores a template or copy of the genetic information ready for use in case of damage of the other strand. If small or gross mutational events affect important functional portions of the genetic material, repair mechanisms can restore the exact sequence of the damaged or lost or deleted parts through restoration mechanisms based on the complementary information of the other strand. Mutations leading to damage of corresponding segments of both strands represent a catastrophic event to the genome due to the absence of the sequence database needed for the repair mechanism to define the exact base sequence of the newly synthesized segment in place of the deleted or grossly damaged segment.

### 10.3. Degeneracy of the genetic code

Degeneracy of the genetic code represents the third innate anti-mutation mechanism of the human genome. This feature permits the occurrence of same-sense point mutations in functional codons without changing the amino acid defined by the mutated codon. Since some amino acids, as a part of a



specific protein domain, play critical roles in attaining and maintaining correct protein structure and in mediating proper protein function, point mutations leading to replacement of these essential amino acids by other amino acids, missense mutations, that can't perform the functions of the original amino acids might result in detrimental effects on the structural integrity and stability of the protein followed by deleterious consequences on physiological function of the protein. Hence, degeneracy of the genetic code allows for occurrence of many point mutations, the commonest type of mutational events and the commonest cause of genetic disorders, without changing the final structure of the synthesized protein, thus protecting against, and obviating, the pathological effects of these mutations.

#### 10.4. Nuclear localization of DNA

The localization of DNA deep inside the cell nucleus represents a fourth innate anti-mutation mechanism of the human genome because it acts as a physical barrier against many mutagens that have to overcome many obstacles of cellular defense mechanisms in order to affect the nuclear genome. These defenses include the extra-cellular environment, the cell membrane, the cytoplasmic mass, the cytoplasmic enzymes and phagocytic cellular organelles and the cytoplasmic and nuclear antioxidant enzyme systems.

#### 10.5. DNA-associated proteins

The DNA-associated or DNA-binding proteins, in addition to their essential roles in regulating transcriptional processes of most genes, also play fundamental roles in protecting the DNA from the damaging effects of many mutagens, in particular the free radicals that are generated during metabolic activities of the cell. They act as physical barriers and biochemical buffers, modifiers or deactivating biomolecules of many chemical mutagens or damaging factors that might harm the DNA. They mediate this protective role by many mechanisms including modulation of charge transport of oxidative agents within the DNA, limitation of DNA helix distortion and regulation of protein-dependent alterations in DNA base stacking [1].

#### 10.6. Replication proofreading system

Preservation of genomic identity of the organism depends exclusively on accurate replication and synthesis of two identical copies of the genome during cell division, followed by transfer, or inheritance, of each copy to each daughter cell. In this manner, all cells descendent from a parent cell have nuclear genomes identical to those of the mother cell. The majority of spontaneous point mutations of the nuclear genome are prone to occur during cell division, mostly during DNA synthesis or the replication phase of the process. The replication proofreading system acts in a prophylactic way to ensure accurate insertion or addition of the proper nucleotide to the newly synthesized strand of replicating DNA. This prophylactic function is fundamental to reduce the rate of inevitable replication mistakes to minimum levels that could be dealt with efficiently with the DNA repair mechanisms. In spite of the impressively fast and accurate ability of the enzymes

responsible for DNA synthesis, the DNA polymerases, most of them have additional proofreading ability to ensure accurate error-free DNA replication and, hence, maintaining and preserving the stability, integrity and identity of the genome during cell division, as well as during transfer of the genetic material from parents to offspring.

#### 10.7. Genetic repair systems

Genetic repair systems responsible for correcting and repairing many different types of point and small mutations that affect the genetic material, whether induced by exogenous mutagens or occurring secondary to endogenous spontaneous alterations, comprise both nuclear DNA repair system and mitochondrial DNA repair system. Genetic function and genetic repair represent two sides of one coin. Without the persevering continuous, active and effective surveillance exerted by the genetic repair systems to detect and repair the continuously and persistently occurring mutations, maintaining stability and integrity of the genome would be an impossible task. These repair systems consist of large numbers of enzymes, proteins and related factors that function in complementary and collaborative mechanisms along specific pathways, with each of them having a predefined role in the repair process. For instance, if mutation causes damage of a genetic segment consisting of sequence of nucleotides, an endonuclease enzyme cuts both sides of the damaged segment, followed by addition of proper nucleotides instead of the damaged or deleted ones by a polymerase enzyme, then a ligase enzyme joins the ends of the newly added segment of nucleotides to the original neighboring nucleotides by forming phosphor-di-ester bonds between the phosphate and the sugar of adjacent nucleotides, thus, regaining the sugar-phosphate backbone of the DNA [2].

##### 10.7.1. Nuclear DNA repair

Nuclear DNA repair mechanisms comprise many approaches to repair mutations of DNA. These approaches include different pathways and sub-pathways according to the type, site and extent of the mutation-induced damage and also according to the stage of cell cycle affected by the mutation. They include: base excision repair (BER), nucleotide excision repair (NER), direct reversal repair, mismatch repair, transcription-coupled repair (TCR) and recombination repair (Fig. 11).

**10.7.1.1. Base excision repair (BER).** This repair mechanism is probably the most frequent DNA repair pathway in the cell. It is used for single strand point mutations affecting one or few bases of one DNA strand. It involves recognition of the damaged base of a nucleotide by a glycosylase enzyme and its removal by detaching it from the deoxyribose sugar via hydrolysis of the N-glycosyl bond. Breakage of the hydrogen bond between the damaged base and the opposing base on the complementary strand occurs, probably, via ATP-induced changes of the energy dynamics of the bond. Removal of the damaged or mutated base results in creation of abasic site or apurinic/apyrimidinic site (AP) of the DNA which are targeted by endonuclease and lyase activity to remove the damaged base(s) followed by addition of new normal base(s) by a specific polymerase enzyme and, finally, regaining the phosphodi-ester bonds and, hence, the phosphate-sugar backbone of the DNA strand by the action of DNA ligase.

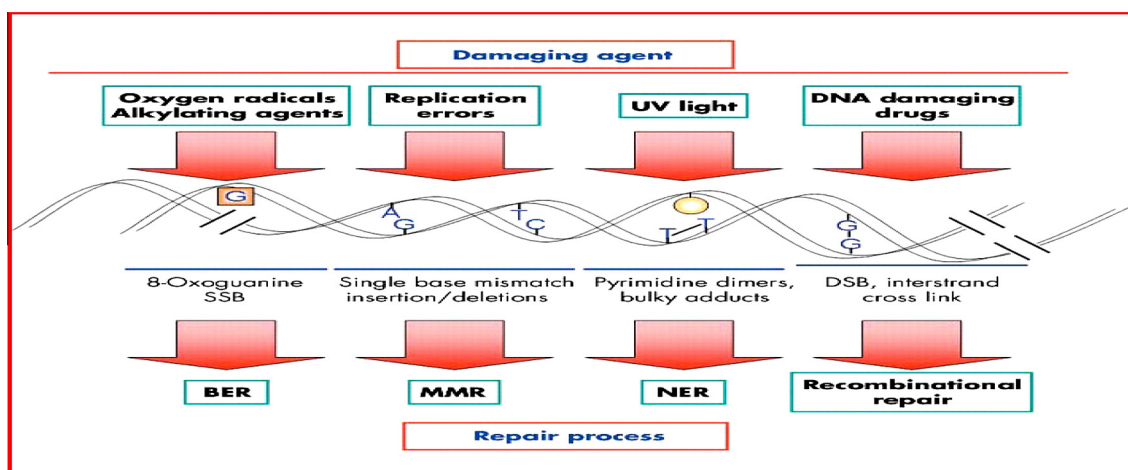


Figure 11 DNA repair mechanisms.

**10.7.1.2. Nucleotide excision repair (NER).** Nucleotide excision repair is one of the most important DNA repair systems and is highly conserved among species, though it is much more complicated in higher eukaryotes than prokaryotes. The most prominent feature of this repair system is its broad substrate specificity because it can excise DNA lesions such as UV-induced pyrimidine dimers as well as more bulky adducts of DNA.

**10.7.1.3. Direct reversal repair.** This repair mechanism can directly repair UV-induced pyrimidine dimer formation and alkylation adducts by DNA photolyase enzymes and alkyltransferase proteins, respectively. Direct reversal repair mechanisms are not followed by incision of DNA strands or resynthesis of new DNA since the changed or mutated bases are directly reverted to their original states either by light-dependent photoreactivation process, for pyrimidine dimers repair, or by use of alkyltransferase. Placental mammals do not have photolyase-dependent repair mechanisms and depend on nucleotide excision repair to correct and repair UV-induced pyrimidine dimer formation.

**10.7.1.4. Mismatch repair (MMR).** The mismatch repair (MMR) system recognizes and corrects mismatched or unpaired bases that result from errors of DNA polymerase during DNA replication. It involves complex reactions and interactions of many enzymes, proteins and signal discrimination factors, probably in collaboration with the proofreading system, to recognize the mutated strand first and then to locate the site of the mismatched pair. This is followed by removal of the mutated sequence by an endonuclease, addition of new pair(s) by DNA polymerase and final regain of the DNA double stranded structure by DNA ligases. Post-replication mismatch repair is achieved by removal of a relatively long tract of mismatch-containing oligonucleotides, a process called long-patch MMR.

**10.7.1.5. Recombination repair.** Recombination repair mechanisms aim primarily at repairing double-strand breaks of DNA which represent the most devastating mutation-induced lesions of DNA because they can lead to loss of genetic information and chromosomal instabilities with consequent pathological alterations including chromosome breakage syndromes and carcinogenesis. Double-strand breaks can

be caused either endogenously during DNA replication due to replication errors, e.g. replication fork collapse, or exogenously by, e.g. ionizing radiation. Recombination repair mechanisms consist of many various steps: end resection, strand invasion, DNA repair synthesis, branch migration and Holliday junction resolution, and include, at least, two different repair pathways: homologous recombination repair (HR) and nonhomologous end-joining repair. HR repair mechanism is the accurate pathway and makes use of undamaged homologous DNA as a template for repair. Non-homologous end-joining repair mechanism directly ligates two double-strand break ends together, and although it is efficient, it is prone to loss of genetic information at the ligation sites. However, there are many anti-recombination mechanisms to suppress excessive recombination that might lead to loss of genetic information and genomic instability [1].

#### 10.7.2. RNA repair/editing system

RNA editing refers to molecular modifications of nucleotides of RNA through chemical changes in the base makeup of the molecule. Such changes appear to be present in all three domains of life, and involve both coding, mRNA, and non-coding, tRNA and mRNA, types of small or microRNA. RNA editing occurs in the cell nucleus and the cytosol, as well as in mitochondria and is mediated by a complex repair system comprising many species of small RNA (guide RNA) and large protein complexes known as the editosomes. The pathways of RNA editing include many diverse processes: nucleoside base modifications such as cytidine (C) to uridine (U) and adenosine (A) to inosine (I) deaminations, as well as non-templated insertions of nucleotide. RNA editing in mRNAs effectively alters the amino acid sequence of the encoded protein so that it differs from that predicted by the genomic DNA sequence. Though mRNA editing is used in many instances to allow for synthesis by the cell of more than one protein from the same mRNA transcript, e.g. synthesis of both apolipoprotein B-100 and apolipoprotein B-48 from the same mRNA in liver cells, it can also be used to repair missense or termination mutations of the molecule which can have deleterious effects on the synthesized protein. Specific endonucleases and ligases for double stranded species of RNA have been defined in many prokaryotes and it might be just a matter of time before defining their functional counterparts in eukaryotes and human cells.

### 10.7.3. Mitochondrial DNA (mtDNA) repair

The pivotal role played by the mitochondrial genome in generating ATP, without which life can neither begin nor persist, in addition to the many other critical metabolic and regulatory functions of mitochondrial genes, requires the presence of an efficient system for repairing mtDNA mutations. The need for mitochondrial genome repair system is further imposed on the cell in view of the high mutation rate of mitochondrial genes which lack many of the anti-mutation and protective mechanisms available to nuclear genes. Similar to the nuclear genome repair system, mitochondrial repair system includes many repair pathways: base excision repair, direct reversal repair, mismatch repair, and recombination repair. Nucleotide excision repair (NER) pathway, however, seems not to be working in the mitochondria [12].

### 10.8. Protein repair systems

Accurate post-translation structural configuration of newly synthesized polypeptide chains is a fundamental conformational modification for most proteins to become functionally active biomolecules. The maturation from primary to quaternary protein structure involves many changes, e.g. folding and maintenance of steric and spatial relationships between the different domains of the protein. Conformational defects in proteins that might happen during these modifications can lead to the formation of misfolded and/or aggregated non-functional molecules.

Human genome comprises a large number of genes that code a complex system composed of large numbers of specific protein families and subfamilies known as molecular chaperones. These proteins have many important and diverse functions in cellular activities, e.g. assisting non-covalent folding or unfolding and assembly or disassembly of macromolecular structures, including proteins. Prevention of misfolding and/or aggregation of newly synthesized polypeptide chains, which turn them to nonfunctional biomolecules, is a major and fundamental function of molecular chaperones. Other physiological functions of chaperones include: transport across mitochondrial membranes and the endoplasmic reticulum and assistance in protein degradation.

Molecular chaperones, probably, exert critical roles in maintaining stability and integrity of the proteome. This state of protein homeostasis, proteostasis, is a prerequisite for proper control and regulation of cellular metabolic networks by proteins and is mandatory for efficient mediation of cellular activities. Specific species of molecular chaperones, surveillance chaperones, are responsible for constant surveillance of the proteome to ensure proper protein homeostasis. Age-related decline or mutation-induced defects in proteome stability and integrity results in progressive aggregation and faulty conformational changes of proteins, both of which are associated with, and underlie, the development of many genetic diseases like Alzheimer disease, Parkinson disease, prion diseases and many others [13].

### 10.9. Silencing of transposon activity during development

Transposons constitute a considerable portion, nearly 40%, of the human nuclear genome. Transposon activities might have contradictory effects on the stability and integrity of the

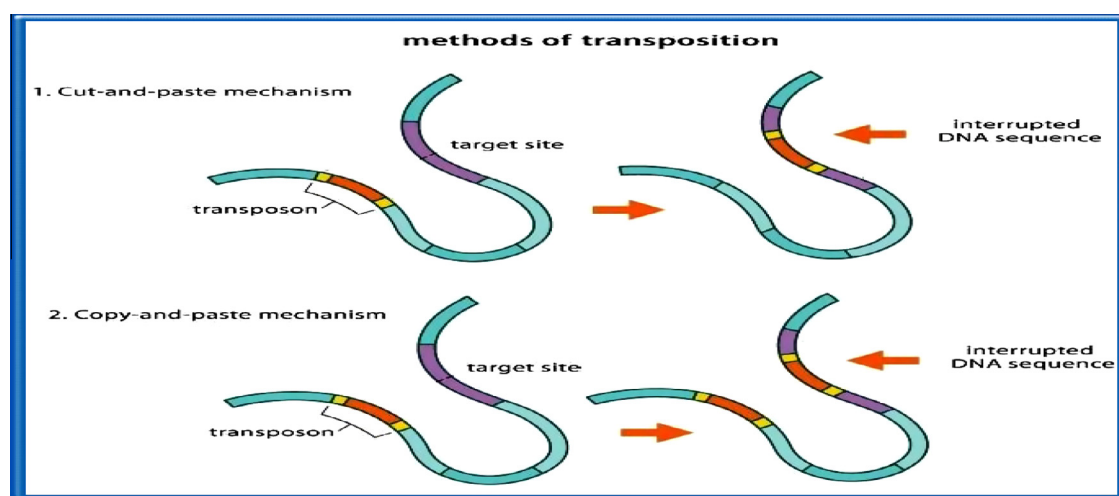
nuclear genome. They might behave in a harmful way and act as major potential causes of spontaneous mutations of the nuclear genome. They can make a copy of themselves and insert the new copy in another site, or they can detach themselves from their location and get inserted at different sites of the genome (Fig. 12). In both conditions they result in insertional mutagenesis with consequent deleterious effects on genomic stability and genomic integrity. If they get inserted in a functional segment of the genome they lead to structural disruption and loss of function of the affected segment with resultant pathological effects. Alternatively, transposon activity may lead to creation and construction of new genetic combinations that may have specific functions. Within this context, they would be considered as one of the genetic biological mechanisms involved in, and responsible for, evolutionary diversity of the genome and the proteome. They can also cause tangible increases in the amount of the genetic material due to recurrent synthesis and addition of multiple new copies of transposable elements to the nuclear genome.

Transposons probably have a central regulatory role in the early development of the embryo. A key feature of this activity involves a specific type of transposable element (MuERV-L) which is uniquely active during the totipotent cell phase of embryogenesis. A network of regulatory genes that are particularly active during early development depends on this transposable element to activate their promoter areas in order to start their transcriptional activities. The early timed enhancement of the regulatory functions of this genetic network seems essential for proper differentiation of the totipotent cells which play fundamental roles in development and differentiation. As development continues, progressive silencing of the genes of this regulatory network takes place. This delicate balance in temporal regulation of transposon activity is critical for embryogenesis and development as well as for proper development of germ line cells, particularly those in spermatocytes during spermatogenesis [14].

Over activity or uncontrolled activity of transposons can, thus, have detrimental and devastating effects on embryogenesis, differentiation and development, and can lead to the pathogenesis of a wide variety of congenital malformations and genetic defects. The human genome, however, has a unique control system composed of a specific subtype of small or micro RNA molecules, known as piwiRNA, or piRNA, composed of RNA-piwi protein complexes. They are thought to be involved in gene silencing, most specifically the silencing of transposons. The majority of piRNAs are antisense to transposon sequences suggesting that transposons are the main target of piRNA. In mammals, the marked activity of piRNAs in silencing of transposons and control of their activities is most important during the development of the embryo in order to reduce the rate and risk of transposon-induced mutations during this sensitive period of life [15].

### 10.10. Antioxidant enzyme systems

The continuous functioning of the exceedingly huge number of metabolic networks that mediate cellular activities in living cells results in continuous generation of many different types of useful and harmful metabolic by-products. Oxidant free radicals constitute one of the most crucial categories of these by-products in view of their ability to induce widespread damage



**Figure 12** Transposons: methods of transposition. (Lauren Solomon, Broad Institute of Harvard and MIT),

in many cellular components including membranes, organelles and structural macromolecules like lipids and proteins. This structural damage, unless counteracted by opposing antioxidant mechanisms, results in progressive degradation of cellular constituents with consequent resultant pathophysiological alterations of cellular functions, leading ultimately to disease. Although low concentrations of reactive oxygen species may be beneficial, or even necessary, in mediating many cellular processes, e.g. defense against invading micro-organisms and intracellular signaling pathways, nevertheless, higher concentrations of these free radicals play a causative role in the aging process as well as in the pathogenesis of many human disease states, including immune deficiency, neurodegeneration and cancer. Oxidative damage of nDNA, mtDNA, RNA and DNA-binding proteins by free radicals represents an important category of detrimental genetic mutations induced by endogenous chemical mutagens inevitably generated during cellular metabolic activities and other cellular functions.

Living cells have several efficient non-enzymatic and enzymatic antioxidant activities that are responsible for eliminating and/or terminating the chain reactions following generation of free radicals, as a safeguard against their damaging effects on cellular components and cellular functions. Enzymatic antioxidant systems of the cell comprise large numbers of multiple types of antioxidant enzymes, notably catalase, superoxide dismutase, thioredoxin reductase, glutathione peroxidase and various other peroxidases. Efficient production of these antioxidant enzymes and proper regulation of their functions is mandatory to keep and maintain redox homeostasis of the cell which is a critical prerequisite for normal mediation of cellular activities [16].

#### 10.11. Apoptosis of heavily, lethally mutated cells

Apoptosis, or programmed cell death, is a universal biological behavior of most living cells necessary, in conjunction with other life-regulating mechanisms, for maintaining the vital balance between life and death that governs optimal life conditions of multicellular organisms. Apoptosis plays fundamental and crucial roles in normal growth and development as well as in normal differentiation and determination of the proper final architecture of cells, tissues and organs. Faulty timing or

incorrect accomplishment of specific and selective apoptotic processes during each of these life stages of the cell might results in devastating consequences on cellular functions that range from dysfunction to malformation, and may, even, culminate ultimately in premature cell death and pathogenesis of disease [17].

Apoptosis plays a crucial role in maintaining genomic stability and integrity, not of individual cells, but of the organism as a whole. Induction of apoptotic mechanisms in heavily mutated or lethally mutated cells leads to death of the cell and prevents transfer of these mutations to its putative descendant daughter cells. This fundamental prophylactic anti-mutation role of apoptosis in cellular activities and life prospects of living organisms has more far-reaching effects on many important aspects related to the balance between, and the incidence of, normal and mutant genotypes within species-specific gene pools. Additionally, apoptosis can affect in an appreciable manner genomic identity of living organisms because mutation-induced evolutionary or decadence pathways are largely dependent on the outcome of certain apoptotic mechanisms operating during certain stages of the cell cycle.

#### 10.12. Melatonin

Melatonin is a hormone synthesized by the pineal gland, bone marrow cells, epithelial cells and lymphocytes. Melatonin receptors are distributed in most organs, a finding reflecting its widespread roles in regulating various physiological and psychological processes. Many in vitro and animal studies revealed that melatonin has diverse functions including effective protection of cells against radiation-induced chromosome breakage and inhibition of tumor development in animals exposed to experimental chemical carcinogenesis [18]. Melatonin was shown to have protective effect against oxidative DNA damage by chemical inactivation of DNA-damaging agent as well as by stimulating DNA repair mechanisms. These anti-mutagenic and anti-clastogenic effects of melatonin can be linked with its ability to protect DNA against oxidative damage. It may exert this antioxidant action by eliminating harmful reactive oxygen radicals or by stimulating the repair processes of oxidative stress-induced damage of DNA [19] [Table 2].



**Table 2** Anti-mutation mechanisms of the human genome.

Anti-mutation mechanisms of the human genome	
Mechanism	Types & pathways & comments
1. Structural organization of the genome	1. Nuclear genome
2. Structural features of DNA	2. Mitochondrial genome
3. Degeneracy of the genetic code	Complementary strand stores genetic information
4. Nuclear localization of DNA	Multiple point mutations might occur without affecting synthesized protein
5. DNA-associated proteins	Physical protection of nuclear genome
6. Replication proofreading system	1. Physical barriers
7. Genetic repair systems	2. Biochemical buffers
	3. Deactivating biomolecules
	4. Modulation of charge transport
	5. Limitation of DNA helix distortion
	Prophylactic pathway during DNA replication
	A. Nuclear DNA repair
	1. Base excision repair
	2. Nucleotide excision repair
	3. Direct reversal repair
	4. Mismatch repair
	5. Recombination repair
	B. RNA repair/editing system Post-transcription repair/editing of some mRNA defects via guide RNA (gRNA)/editosome complex
	C. Mitochondrial DNA (mtDNA) repair
8. Protein repair systems	Correction of post-translation protein misfolding/aggregation by chaperones
9. Silencing of transposons by piwiRNA	Reduces transposon-induced mutations during development
10. Antioxidant enzyme systems	
11. Apoptosis	Prophylactic pathway against spread of mutations of heavily or lethally mutated genomes to daughter cells
12. Melatonin	Anti-clastogenic, anti-mutagenic, anti-carcinogenic and anti-oxidant compound

## 11. Genetic imprinting

Among known pathogenetic mechanisms implicated in development of genetic disorders, the phenomenon of genetic imprinting represents one of the most puzzling phenomena in genetics. Genetic imprinting refers to differential functional expression/repression of chromosomes, parts of chromosomes, single genes or sets of genes. The differential expression/repression of imprinted component(s) depends on either the parent of origin (parental imprinting), the adjacent DNA-associated proteins (spatial imprinting) or the timing of post-fertilization development (temporal imprinting). Though pathogenetic mechanisms caused and mediated by defective temporal and spatial imprinting are self-explanatory, those due to parental imprinting are, still, unexplained. Imprinting probably represents one, among many, species-specific innate genomic mechanisms responsible for the establishment of the cardinal fundamental aspects of genomic identity including the formulation of characteristic species-specific profiles of the transcriptome and the proteome and the development of sexually distinctive phenotypes. Though current observations point to the possible roles of microRNA and chromatin components in mediation and maintenance of imprinting processes through biochemical molecules/networks, imprinting might be mediated by other different unexpected mechanisms apart from the currently identified conventional biochemical mechanisms, e.g. the geomagnetic imprinting postulation [20].

## 12. Biological consequences of mutation

### 12.1. Mutation and genetic adaptations to stress

Although the development and appearance of new biological functions secondary to environmental effectors or endogenous mutational events reflect the concept of adaptation, a fundamental and universal biological principle shared by living organisms and indispensable for any biological systems existing in a continuously varying and stressful environment, adaptation needs not be considered as modifications secondary to genomic alterations. As referred to previously, the concept of evolution is hard to consider unless solid evidence of progressive and appreciable increase in proteome size accompanied by parallel increase in genome size from simple to more complex biological systems, e.g. from unicellular to multicellular organisms, is documented. Actually, this is not the case as many simple organisms have larger genomes than more complex organisms. This might be interpreted by more liability of simple organisms, which have simple cellular architecture and deficient protective mechanisms, to infection by invading viruses capable of amalgamating their DNA with host DNA with consequent increase of size of host genome, compared to more complex organisms that have protective and anti-mutation mechanisms against infecting viruses.

Reconsidering the proteome as being a separate and independent biological system participating in defining life framework of living cells, not merely a tributary structured system

synthesized under regulatory control of the genome, might help in interpreting some perplexing aspects of mutation-induced evolutionary adaptation. Duplication of the genetic material, either on gene level or chromosome level, is looked at as evolutionary solution to stress and evidence of their occurrence in yeast has been revealed in many studies [21]. Increased genome size in response to stressful conditions, nevertheless, should be induced first by deficiency in proteome functions because stressful environmental stimuli exert their unfavorable impact first on the metabolic networks in the cytoplasm or in other cellular components. They do not affect the genome in a straightforward way except under certain extraordinary conditions, e.g. direct damage to genes. Neither qualitative nor quantitative changes of the proteome have direct or lasting effects on constitution of the genome in a manner capable of compelling the genome to respond in an adaptive way leading to duplication of some of its components. Compensatory increase of gene product in response to stressful conditions can be attained via increasing gene expression by many known mechanisms, e.g. enhancing transcription through promoter activation, increasing stabilization of mRNA and multiple translation rounds of the same transcript, without need to increase genome size by gene or chromosome duplication. Modifications of DNA-associated histones, for instance, have both enhancing and silencing regulatory effects on concerned genes but they do not result in, or induce, genetic duplications or increase in genome size.

### 12.2. Mutation and evolutionary variation

In spite of the exquisite ability of the protein translation system in recognizing and decoding the mRNA transcript and harmonizing the actions of tens to hundreds of factors involved in protein synthesis, it can't recognize changes of the original genetic code embodied within the codon sequence of the mRNA transcript. It decodes and recognizes triplets of bases, or codons, along the transcript without giving attention to whether they are comparable and complementary to the original sequences of the gene or not. It seems that the protein translation system has no prior memory to predict the validity of the codon sequence of the mRNA transcript in respect to both the gene sequence and the amino acid sequence of the protein. Though this defect of the translation system might be considered as a prerequisite for evolutionary variation of protein phenotypes necessary for acquisition of new functional abilities, e.g. formulation and construction of new metabolic pathways, it is a major cause of pathogenesis of genetic defects due to the absence of a translation proofreading and repair system in comparison with the DNA and mRNA repair systems. Absence of genetic memory necessary for proofreading of translated proteins is enigmatic and bewildering in view of the prime and critical significance of the translation process, since the majority of genetic diseases result from synthesis of defective proteins or deficient synthesis of required proteins. Though it might be considered as a genomic regulatory mechanism allowing for selective pressure to proceed in favor of evolutionary variation, it results in marked pathological effects on the organism if newly synthesized proteins are structurally defective, a finding that poses the concept of biological evolution in direct contradiction with our current knowledge regarding the structural and functional characteristics of both

the genome and proteome. Stressful environmental conditions, traditionally considered as the main triggers of evolutionary adaptations, affect the proteome of the cell first. Accordingly, if assumptions regarding genomic adaptations as evolutionary processes are postulated, they have to reveal first how primary disturbances in structure or function of the proteome can lead to adaptive changes in structure and function of the genome. This feature is not to be confused with protein repair mechanisms conducted by the chaperones family of proteins which have the ability to correct some aggregation and misfolding defects of already synthesized proteins, in addition to many other important cellular functions, but have nothing to do with decoding and recognition defects during the actual process of translation and protein synthesis [22].

Though the possibility of formulating novel, meaningful metabolically active networks composed of, and mediated by, new functioning proteins synthesized as a result of defective translation, is extremely difficult to accept or to interpret by the basic concepts of randomness and/or coincidence, its occurrence will be temporally limited by the life cycle of the cell. Unless adaptive genomic changes creating new genes coding for the new proteins occur, newly formulated networks can't be maintained, fixed or inherited. Some sort of interactive feedback mechanism between the proteome and the genome, similar to classic stimulus-response pathways, might exist and represent the missing link mediating this proteome-genome interaction. However, these assumptions rely, basically, on the hypothesis of participation of the proteome, as a separate and independent regulatory structured system, in conducting life processes in the cell and in inducing adaptive and sustained changes of the genome. Alternatively, supporters of the theoretical hypothesis which postulates that evolution and divergence could be initiated, mediated and maintained, primarily, by genomic adaptation in response to stressful or demanding environmental conditions have to reveal induced mutagenic beneficial effects capable of causing increases in genome size. Currently known mutagens, e.g. irradiation-chemicals-viruses, are damaging agents and result in detrimental effects and pathogenetic consequences leading, in most instances, to disease.

### 12.3. Mutation and natural selection

The concept of mutation-induced natural selection might be applicable in nature but not in life. The continuing occurrence and persistence of genetic defects deleteriously affecting biological fitness, as measured by survival and reproduction, among humans contradicts assumed roles attributed to natural selection as a major effector of adaptation and evolution. Mutations that cause these genetic defects occur spontaneously at a more or less constant rate among most populations. Eradication or lessening their harmful burden can be achieved only by targeted, rather than by natural, selection, e.g. through pre-implantation diagnosis, prenatal detection and termination of pregnancy and other similar prophylactic genetic measures. In the animal kingdom, for instance, there is no chance for survival or reproduction of sick or weak animals, similarly in the plant kingdom, plants incapable of tolerating conditions of dehydration due to accidental lack of water would perish quickly, but this is not the case in human life where deeply rooted spiritual considerations rule and control

the way we manipulate and control our genomes, not the reverse. There is no place for the concept of mutation-induced natural selection in human life or in human genetics. Instead of that, the concept of creative or artificial selection, e.g. cloning and trials to construct new creatures with new genomes, in spite of moral protestation and ethical expostulation against its applications, will probably accomplish the major role in inducing true evolutionary changes of the human genome through targeted reproductive mechanisms like pre-marital, pre-implantation and prenatal diagnosis.

### Conflict of interest

We have no conflict of interest to declare

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